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U. S. DEPARTMENT OF AGRICULTURE.

BUREAU OF PLANT INDUSTRY—BULLETIN NO. 246.

B. T. GALLOWAY, *Chief of Bureau.*

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# THE RELATION OF BARIUM TO THE LOCO-WEED DISEASE.

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## I.—A FIELD STUDY ON THE RELATION OF BARIUM TO THE LOCO-WEED DISEASE.

BY

C. DWIGHT MARSH,

*Physiologist, Drug-Plant, Poisonous-Plant, Physiological,  
and Fermentation Investigations.*

## II.—LABORATORY STUDIES ON THE RELATION OF BARIUM TO THE LOCO-WEED DISEASE.

BY

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*Chemical Biologists, Drug-Plant, Poisonous-Plant, Physiological,  
and Fermentation Investigations.*

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## LETTER OF TRANSMITTAL.

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U. S. DEPARTMENT OF AGRICULTURE,  
BUREAU OF PLANT INDUSTRY,  
OFFICE OF THE CHIEF,  
*Washington, D. C., February 15, 1912.*

SIR: I have the honor to transmit herewith and to recommend for publication as Bulletin No. 246 of the series of this Bureau the accompanying manuscript, entitled "The Relation of Barium to the Loco-Weed Disease." The manuscript consists of two separate contributions to the knowledge of the loco-weed disease, written from different standpoints: (1) "A Field Study on the Relation of Barium to the Loco-Weed Disease," by Dr. C. Dwight Marsh, Physiologist, and (2) "Laboratory Studies on the Relation of Barium to the Loco-Weed Disease," by Dr. C. L. Alsberg and Mr. O. F. Black, Chemical Biologists.

These investigations were carried out in the Office of Drug-Plant, Poisonous-Plant, Physiological, and Fermentation Investigations under the direction of Dr. Rodney H. True, Physiologist in Charge. The first paper constitutes a contribution to the study of the loco-weed problem as encountered by stockmen in many of the grazing States of the West; the second paper presents evidence gained through the help of the laboratory. In this connection, information obtained from the barium-bearing regions in Virginia is used to shed light on the situation. A full bibliography of barium poisoning, prepared in connection with the first paper, is kept on file for future reference.

Respectfully,

B. T. GALLOWAY,  
*Chief of Bureau.*

HON. JAMES WILSON,  
*Secretary of Agriculture.*



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# THE RELATION OF BARIUM TO THE LOCO-WEED DISEASE.

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## I.—A FIELD STUDY ON THE RELATION OF BARIUM TO THE LOCO-WEED DISEASE.

By C. DWIGHT MARSH, *Physiologist, Drug-Plant, Poisonous-Plant, Physiological,  
and Fermentation Investigations.*

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### INTRODUCTION.

The work of Dr. Albert C. Crawford,<sup>1</sup> formerly Pharmacologist in the Bureau of Plant Industry, in determining the presence of barium in loco weeds and his conclusions that, at Hugo, Colo., at least, the inorganic constituents, especially barium, are responsible for the symptoms of loco poisoning gave a logical basis for experimental work on antidotes. Inasmuch as his work was carried out under laboratory conditions and on animals different from those that commonly suffer from loco poisoning, it was necessary for the confirmation of his results to make feeding experiments with barium on cattle and horses under range conditions. This report deals with those two phases of the field work of 1908.

In order that an antidote may be of practical use it must be cheap and must be capable of being administered in such a way as to meet the conditions of range animals. Anything like individual treatment is, of course, impracticable. The question was naturally raised whether an antidote could not be given in water or in salt. In a large part of the West animals are watered at definite places, and it would be possible to apply a cheap antidote through the water, if such an antidote could be found, and thus counteract the effects of the poison; or, if it were possible to mix the antidote with salt, it is evident that it could be so administered.

### AMOUNT OF BARIUM TAKEN IN DAILY DOSES IN LOCO FEEDING.

In order to compute the amount of antidote necessary it is important to have at least an approximate idea of the quantity of barium

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<sup>1</sup> Crawford, A. C. Barium, a Cause of the Loco-Weed Disease. Bulletin 129, Bureau of Plant Industry, U. S. Dept. of Agriculture, 1908, p. 75.

that an animal will get in an average day's feeding of loco. Crawford bases his estimate of the amount of barium ingested daily on the assumption that the daily ration of grass is 60 pounds.<sup>1</sup> Other authors than those quoted by him make the daily ration much less. For example, Jordan<sup>2</sup> states that the maintenance ration of mixed hay for bovines is 15.5 pounds per day. It is very difficult to find authoritative statements in regard to the quantity of grass eaten daily by the average horse or steer. Jordan's estimate has been considered by the writer as a minimum and Crawford's as a maximum, and the experimental work was carried out on the basis of both estimates. Using Jordan's estimate, if it is assumed that the grass of the western plains weighs twice as much as hay, the daily ration of grass is about 30 pounds. As range animals feed on comparatively dry grasses, this is probably an overestimate. It is impossible to say what proportion of this daily ration would be loco. Post-mortems show that all loco eaters take a large proportion of other food. It is rarely true that an animal eats loco exclusively. Chronic cases gradually eat less and less until they starve to death. Hence, the assumption that half of the daily food is loco would doubtless be a large overestimate. On the basis of a daily ration of 30 pounds, assuming that half of the food is loco, an animal would eat 15 pounds, or about 8 kilograms, of the weed daily. Crawford estimates<sup>3</sup> that 12.44 per cent of the dried plant is ash<sup>4</sup> and in this ash 1 gram has 0.0026 of barium sulphate.

Assuming that an animal will eat as much as 8 kilograms (17.64 pounds) of loco daily, this would be equivalent to a little over 5 kilograms (11 pounds) of dry material, inasmuch as loco on the average loses about one-third when drying in the air, and 12.44 per cent of these 5 kilograms would be the ash in the daily food. Calling it for the sake of round numbers 12.5 per cent, the ash in the daily food would be about 625 grams. Inasmuch as there is 0.0026 gram of barium salts in 1 gram of the ash, the daily feed of loco would contain 1.625 grams of barium salts. If, on the other hand, Crawford's estimate of the daily ration is taken and the assumption is made that an animal on a full daily diet of loco would get about 10 grams of barium salts and if one assumes that of this daily diet half is loco, the animal would then get 5 grams of barium salts daily. In arranging for the experiment, therefore, on the basis of Crawford's analyses, it appears that one might assume that an animal would get in its diet a maximum daily allowance of barium salts of 2 to 5 grams.

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<sup>1</sup> Crawford, A. C. Op. cit., p. 74.

<sup>2</sup> Jordan, W. H. The Feeding of Animals, 2d ed., New York, 1903, p. 299.

<sup>3</sup> Crawford, A. C. Op. cit., p. 75.

<sup>4</sup> The loco weed used in these series of experiments was *Aragallus lamberti*, all gathered near Hugo, Colo.

### ANTIDOTES USED.

Magnesium sulphate was the substance naturally thought of as an antidote, as it is a recognized remedy for barium poisoning, is easily obtained, and is cheap. Consequently the experiment was mainly in the use of this substance. It was thought best also to try sulphuric acid, as it, too, would be a logical antidote and is cheap.

### POSSIBILITY OF GIVING ANTIDOTES IN SALT.

Horses upon the plains of Colorado eat so little salt that it is evident that an antidotal effect would not be assured by mixing Epsom salts with their salt. In regard to cattle, Henry states that a steer or cow takes about 1 ounce of salt daily. An ounce avoirdupois is equal to 28.297 grams. If it is assumed that an equal amount of a sulphate, like magnesium sulphate, will serve as an antidote for the barium and an attempt is made to furnish an antidote with the salt there should be added to the salt one-fifth of its weight of the sulphate.

Epsom salts are quite distasteful to cattle and it was evident from the outset that to make them eat salt in which was mixed as much as one-fifth of its weight of Epsom salts would be impossible. So it was definitely decided early in the experiment that it was impossible to administer the antidote in the salt. Even if the lower estimate of the daily ration were used, assuming that half of this were loco, the animal would get a quantity of barium that would require for its neutralization 1.5 grams of Epsom salts; or, one-twentieth of the salt would have to be Epsom salts. This proportion was found to be impracticable.

### POSSIBILITY OF ADMINISTERING ANTIDOTES IN WATER.

Assuming that an animal drinks 70 pounds of water daily, or 35 kilograms, on the basis of 8 kilograms of loco daily the amount of magnesium sulphate in the water would have to be in the ratio of 1 to 19,500. Or, using Crawford's estimate of the amount that an animal may eat daily and assuming that a steer gets as much as 5 grams of the barium salts, it follows that the animal would have to receive about an equal quantity of the antidote, and to obtain this amount the ratio of the magnesium sulphate to the water would be about as 1 to 7,000. It was found as a matter of experiment that animals would readily drink water containing magnesium sulphate even when the proportions were quite large. Therefore it seemed to be practicable to administer the antidote in this way.

### POSSIBILITY OF USING SULPHURIC ACID AS AN ANTIDOTE.

If it is assumed, as in former estimates, that an animal gets 1.625 grams of barium salts daily, the required antidote would be about 0.773 gram of sulphuric acid. These figures are based on the estimate

of a daily ration of 8 kilograms of loco. If Crawford's estimate is taken, the animal would receive 5 grams of barium salts daily. To neutralize these salts would require about 2.4 grams of sulphuric acid. It would be entirely practicable to give this or even very much larger doses in the drinking water.

#### ACTUAL AMOUNT OF BARIUM IN THE LOCO PLANTS FED.

Crawford reports wide variations in the published analyses of loco. Therefore it seemed important to have the loco plants actually used analyzed for their barium content. The plants fed during the summer's experiment were collected from several localities near Hugo, Colo. The analyses made by the Bureau of Chemistry are shown in Table I.

TABLE I.—*Amount of barium in loco plants used in feeding experiments.*

| Plant No. | Ash.             | Barium<br>in ash. | Plant No. | Ash.             | Barium<br>in ash. |
|-----------|------------------|-------------------|-----------|------------------|-------------------|
|           | <i>Per cent.</i> | <i>Per cent.</i>  |           | <i>Per cent.</i> | <i>Per cent.</i>  |
| 8.53      | 42.67            | 0.023             | 8.30      | 22.08            | 0.106             |
| 8.16      | 21.05            | .11               | 8.15      | 17.01            | .017              |
| 8.18      | 10.78            | .088              | 8.96      | 29.96            | .033              |
| 8.29      | 22.16            | .128              | 8.97      | 14.60            | .0683             |

It would appear from Table I that the average ash content of these loco plants was 22.54 per cent and that the average percentage of barium in the ash was 0.0717. Computed in a form showing the amount of barium salts in air-dried loco, it is seen that 1 gram of air-dried loco contains an equivalent of 0.00027 gram of  $\text{BaSO}_4$ , or 0.00029 gram of  $\text{BaCl}_2 + 2\text{H}_2\text{O}$ , or 0.00032 gram of  $\text{Ba}(\text{C}_2\text{H}_3\text{O}_2)_2 + \text{H}_2\text{O}$ .

It was found as a matter of experiment that on the average the loco plants used in Hugo lost one-third in drying in the air. It would appear, therefore, that 1 gram of fresh loco would contain 0.00018 gram of  $\text{BaSO}_4$ , or 0.00019 gram of  $\text{BaCl}_2 + 2\text{H}_2\text{O}$ , or 0.00021 gram of  $\text{Ba}(\text{C}_2\text{H}_3\text{O}_2)_2 + \text{H}_2\text{O}$ .

On the basis of a daily ration of 8 kilograms of fresh loco an animal would get 1.44 grams of  $\text{BaSO}_4$ , or 1.52 grams of  $\text{BaCl}_2 + 2\text{H}_2\text{O}$ , or 1.68 grams of  $\text{Ba}(\text{C}_2\text{H}_3\text{O}_2)_2 + \text{H}_2\text{O}$ . If it ate, according to Crawford, 30 kilograms daily, it would get an equivalent of 5.4 grams of  $\text{BaSO}_4$ , or 5.7 grams of  $\text{BaCl}_2 + 2\text{H}_2\text{O}$ , or 6.3 grams of  $\text{Ba}(\text{C}_2\text{H}_3\text{O}_2)_2 + \text{H}_2\text{O}$ . It would appear from these figures that in the actual experiment, if the lower estimate of the daily ration of fresh loco is used, the animal would consume not over 2 grams of barium salts. If Crawford's estimate of the daily ration is used and it is assumed that half the daily ration is loco, the amount of barium

salts daily ingested would be between 3 and 4 grams. While, therefore, the experiment was started with considerable uncertainty with regard to the actual amount of barium salts concerned, it would appear from the results of these analyses that the actual course of the investigation would have been very nearly the same if the amount of barium in the loco plants which were fed had been known at the beginning of the season.

#### OUTLINE OF EXPERIMENTS ON ANTIDOTES.

In order to control the drinking water, a well was sunk in one of the pastures and the animals were held in such a way that all their water must come from this well. It was planned to pasture a certain number of animals upon loco; to feed definite amounts of weighed loco to others and water some of them at the well with water in which magnesium sulphate had been dissolved; to treat the water for others with sulphuric acid, and also to maintain as a control one or more animals treated with unmedicated water.

The estimates of the amounts of magnesium sulphate and of sulphuric acid necessary were in the first place based upon the assumption that the quantity of sulphates in the water could be ignored. Analyses, however, were made both of the well water and of the water in the creek at which the pastured animals were watered in order to find out the amount of sulphates in those waters. The returns from these analyses, which were made in the Bureau of Chemistry, eventually modified very materially the amount of Epsom salts and sulphuric acid used for antidotal purposes. Hence, in the latter part of the time during which the experiment was conducted, the quantities of magnesium sulphate and sulphuric acid in the water were increased very nearly to the maximum which the animals would take. Care was exercised, of course, that the amount of magnesium sulphate taken should not serve as a physic, but very little trouble was experienced from this source. It was found that the animals would readily drink water with the larger quantity of magnesium sulphate but that they would less readily drink water which was made distinctly acid by sulphuric acid. Crawford<sup>1</sup> has noted the possibility that alkaline bicarbonates in the stomach, which may have been derived from the drinking water, might interfere with the precipitation of barium by the sulphates. This possibility was recognized in the Hugo experiments, but it was assumed that whatever effect the alkaline bicarbonates might have would modify the solubility in only a slight degree and might be ignored in a series of experiments which could not in any case be exact.

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<sup>1</sup>Crawford, A. C. Op. cit., p. 71.

## EXPERIMENTS WITH MAGNESIUM SULPHATE.

On Crawford's estimate that 12.44 per cent of dried loco plant is ash and that this ash has an equivalent of 0.0026 gram of barium sulphate to 1 gram, if it is assumed that an animal eats 8 kilograms of loco daily, the daily feed of loco would contain about 1.6 grams of barium sulphate. This will require rather more than an equal amount of magnesium sulphate to serve as an antidote, or, putting it in round numbers, about 2 grams of magnesium sulphate would be necessary to serve as an antidote to the barium ingested daily. If, on the other hand, use is made of Crawford's estimate that an animal on a full daily diet of loco would get about 10 grams of barium salts presumably calculated as the sulphate, then, on the assumption that half the diet is loco, it would get about 5 grams of barium sulphate. This would take slightly more than 5 grams of magnesium sulphate as an antidote. On this basis it was assumed that in all probability 2 to 3 grams of magnesium sulphate daily would be sufficient to neutralize the probable amount of barium which was taken in connection with the loco feeding.

The experiments were commenced by adding 10 grams of magnesium sulphate to 100 liters (105.7 quarts) of water. On the basis that each animal drinks 25 liters (26.4 quarts), this would give 2.5 grams of the sulphate for each animal. As has been stated before, however, the results of the analysis of water showed that the natural waters near Hugo contain a large percentage of sulphates. On the assumption that an animal drinks 25 liters of water daily, it would follow that inasmuch as the analysis shows that 1 liter (1.057 quarts) of the well water contains 0.3876 of  $\text{SO}_4$ , 25 liters would contain 9.69 grams of  $\text{SO}_4$ . As 9.69 grams of  $\text{SO}_4$  is about equivalent to 25 grams of magnesium sulphate, an animal drinking well water would get the equivalent of 25 grams of Epsom salts daily; or, if it should drink the creek water, it would get 1.65 times as much. Inasmuch as it was known that animals pasturing in the neighborhood of the creek became locoed, it would follow that if the water contained from 40 to 50 grams of magnesium sulphate it would not serve as an antidote. It was evident that if magnesium sulphate was to be used as an antidote, each animal must get something more than 50 grams per day. Because of this fact it seemed desirable to increase the magnesium sulphate to an amount as great as the animal could bear without injury.

The experiment finally was conducted in the following manner: From May 25 to June 1, 10 grams were used to 100 liters of water; from June 2 to 26, 275 grams were used to 100 liters of water; from June 27 to the end of the season, 300 grams were used to 100 liters of water. This last amount evidently would give about 75 grams to

each animal, or including the quantity in the water each animal would get about 100 grams daily. It was found as a matter of experiment that the animals drank the water quite readily, and this experiment was carried on through the season with only a few interruptions on occasions when the animals managed to break through fences and drink creek water.

Four animals were treated in this way, as follows:

Cow No. 35 was treated from May 25 to August 19, eating on the average about 5 pounds daily of green loco. This animal showed distinct symptoms of loco poisoning on August 11, and the experiment was discontinued on August 19.

Steer No. 6 was fed from May 25 to August 31, having a daily ration of about 10 pounds of green loco. This animal was distinctly locoed August 3, and the experiment was discontinued on August 31.

Cow No. 84 was fed from May 25 to September 5. She took on an average about 10 pounds of green loco daily and was distinctly locoed on September 3. This experiment was discontinued on September 5.

Horse No. 57 was an animal which had been partially locoed in 1907, but was improved at the end of that season and did not show many symptoms of loco poisoning in the spring of 1908. This horse was fed from May 27 to August 12, receiving an average of about 8 pounds of green loco daily. It was distinctly locoed on July 29 and died on August 18.

All of these animals were first placed in the corrals and starved for a day or two in order to induce them to eat loco. As soon as the habit was partially formed they were kept in the pasture by day and brought back into the corrals at night, the plan being to give them their feeding of loco at night. If they did not eat readily they were taken out somewhat later in the morning in order to starve them into their doses.

The animals were watered together, so that the amount of magnesium sulphate taken by any animal would depend upon the quantity of water which it drank. In the course of the experiment, however, they averaged pretty close to the estimated amount of 25 liters daily.

It will thus be seen that of the animals which were treated with magnesium sulphate, all became locoed.

#### EXPERIMENTS WITH SULPHURIC ACID.

Basing the work with sulphuric acid on the same figures as in the case of magnesium sulphate, it would appear that an equivalent of 100 grams of magnesium sulphate could be obtained by using 39 grams of  $\text{SO}_4$ , or, assuming that each animal drinks 25 liters daily,

156 grams per 100 liters of water. Inasmuch as the well water contains an equivalent of 38.76 grams of  $\text{SO}_4$  per 100 liters, in order to get the full equivalent of 100 grams of magnesium sulphate each it would be necessary to add to the water 117.24 grams of  $\text{SO}_4$ , or approximately 120 grams of sulphuric acid for every 100 liters. This would be the equivalent of about 66.6 cubic centimeters of the commercial sulphuric acid.

In starting the experiment on June 6 the proportion of sulphuric acid used was 75 cubic centimeters to 100 liters of water. The animals did not drink this proportion readily, so it was reduced somewhat. On June 7, 50 cubic centimeters of sulphuric acid was used to 100 liters of water; from June 14 to 19, 40 cubic centimeters to 100 liters; from June 20 to July 9, 25 cubic centimeters to 100 liters; from July 10 to September 17, 50 cubic centimeters to 100 liters. The reduction in the proportion of sulphuric acid during the intermediate part of the experiment was because most of the animals did not drink the water freely, as the quantity used made the water very distinctly acid, and it was evidently distasteful to them; but as the season progressed, as would be expected, they took the water more freely, and it was found possible to increase the proportion to some extent.

Five animals were fed on measured rations of loco, as in the magnesium sulphate experiments, and watered with the acidulated water, as follows:

Cow No. 31 from June 5 to September 17 received a daily ration averaging between 2 and 3 pounds of green loco, but as a matter of fact she ate very much less, as she refused to eat so large an amount of the weed. She was not affected by the loco.

Steer No. 41 was fed from June 4 to September 25, averaging about 6 pounds of green loco daily. At the end of the experimental period he was in good condition, but showed slight symptoms of loco poisoning.

Bull No. 68 had been partially locoed in 1907, but was in good condition in the spring of 1908. He was fed from June 4 to September 25 an average of 7 pounds of green loco daily. He drank the acidulated water very freely and became distinctly locoed.

Horse No. 55 was fed from May 27 to August 15, receiving an average of about 8 pounds of green loco daily. This animal was started on a mixture of salt and magnesium sulphate, but when that was found impossible, was put with the sulphuric-acid group and received acidulated water from June 6 until the time of death. She was distinctly locoed on August 6 and died August 25.

Cow No. 82 was fed from May 27 to September 25 an average of about 4 pounds of green loco daily. She was watered at the well until

June 6, and from that date received acidulated water. She did not take the loco with great freedom and proved to be an animal somewhat difficult to manage, as she frequently broke through the fence and drank a great deal of water at the creek. She was not affected by loco poisoning.

Of the five animals which were fed upon measured portions of loco and watered with sulphuric-acid water, three became distinctly locoed. If we disregard cow No. 31, as could reasonably be done, inasmuch as she took practically no loco, the result would be that three out of four became locoed.

In addition to the animals which received a measured ration of loco daily, four animals were pastured upon loco and watered at the same time as the preceding group upon water acidulated with sulphuric acid. These animals were as follows:

Cow No. 30 was pastured from May 27 to September 3, receiving the acidulated water from June 18 to the end of the season. She showed some effect of loco poisoning.

Steer No. 33 was locoed in 1907 and cured without treatment other than careful attention to the food. This animal was pastured and watered as in the case of cow No. 30 and became distinctly locoed.

Steer No. 42, locoed in 1907, was the subject of treatment and was considered entirely cured. He was pastured as in the case of the preceding animals and watered with the acidulated water. He showed distinct signs of loco poisoning on July 31 and died on September 9.

Horse No. 75 was in good condition in 1907; was watered with acidulated water from June 18 to July 19. He became very distinctly a loco subject and was placed upon magnesium-sulphate water from July 20 to 24 with the hope of getting beneficial results. After July 24 the antidote treatment was discontinued.

As in the case of magnesium sulphate, the actual amount of sulphuric acid obtained by each animal varied with the quantity of water it drank. This quantity varied more than in the case of the magnesium-sulphate group. No. 82 drank very little of the acidulated water. Some of the time No. 31 drank very little, so that the average for all the animals would be 15 to 20 liters daily. Taking out Nos. 82 and 31, it is probable that the others would average close to the estimated amount of 25 liters daily.

Of the four animals which were pastured upon loco and treated with acidulated water, three became distinctly locoed.

#### CONTROL ANIMALS.

Three animals, as in the preceding cases, were fed measured amounts of loco and were watered with untreated well water, as follows.

Steer No. 43 was fed from May 25 to September 23, receiving an average of  $5\frac{1}{2}$  pounds of green loco daily. It showed no effect of loco poisoning.

Cow No. 91 was fed from May 25 to September 25, receiving an average of 8 pounds of green loco daily. She was distinctly locoed on September 3.

Horse No. 70 was fed from May 27 to August 17, receiving an average of 8 pounds of green loco daily. She became locoed on August 10.

Besides these animals, four were pastured and watered at the well, as in the case of the preceding group.

Cow No. 90 was pastured from May 27 to September 23, receiving creek water up to June 18 and well water from that time on. It was not locoed.

Steer No. 1 was similarly pastured and was not affected by loco poisoning.

Horse No. 72 was pastured from May 27 to July 23, being watered as in the preceding cases, and was distinctly locoed July 20.

It will be seen that of the seven control animals, three receiving a measured ration of loco and four being pastured, three became locoed. In other words, it appears that the animals treated with an antidote became locoed more readily, if anything, than those that received no such treatment.

#### SUMMARY OF RESULTS IN ANTIDOTE WORK.

(1) Granted that barium is the substance producing the symptoms of loco poisoning, the indicated antidotal treatment is the use of the sulphates in some form. Epsom salts is the substance that would most naturally be used, while sulphuric acid would theoretically be just as efficient.

(2) Theoretically, a daily dose of 2 to 5 grams of Epsom salts or of not to exceed 2 grams of concentrated sulphuric acid should be ample to serve as an antidote to the amount of barium ingested in loco feeding upon the range.

(3) Inasmuch as the natural waters where the animals became locoed contain in salts an equivalent of something more than 40 grams of magnesium sulphate in the daily quantity ordinarily drunk by an animal, or something over 12 grams of sulphuric acid, it is evident that more than this proportion must be used to be effective as an antidote, or something over 50 grams of magnesium sulphate or 20 grams of sulphuric acid.

(4) From the results of the experiment it appears that neither magnesium sulphate nor sulphuric acid has any antidotal value in counteracting the effects of loco poisoning.

## EXPERIMENTAL FEEDING WITH BARIUM SALTS.

Crawford in his report<sup>1</sup> makes the following statement:

The main symptoms described in stock on the range can be reproduced on rabbits by feeding extracts of certain loco plants. Those especially referred to here under the term "loco plants," are *Astragalus mollissimus* and *Aragallus lamberti*. \* \* \* The inorganic constituents, especially barium, are responsible for this action, at least in the plants collected at Hugo, Colo. Perhaps in other portions of the country other poisonous principles may be found. A close analogy exists between the clinical symptoms and pathological findings in barium poisoning and those resulting from feeding extracts of certain loco plants. Small doses of barium salts may be administered to rabbits without apparent effect, but suddenly acute symptoms set in analogous to what is reported on the range.

Inasmuch as these experiments had been performed upon rabbits, and the clinical symptoms of loco poisoning had been observed in detail mainly in cattle, horses, and sheep, it seemed necessary, in order to confirm these results and demonstrate that loco poisoning could be produced in cattle, horses, and sheep by barium salts, to make the experiment of feeding barium salts to a number of animals and to have a number of control animals feeding upon loco, making so far as possible the amount of the barium in the two sets of animals equal. In this way it was to be assumed that the symptoms of loco poisoning would be produced in both sets of animals. It may be noted that Crawford<sup>2</sup> states that a part of the barium in the loco plants may not be taken up by the system and that no doubt more of the pure barium salts will be required to produce symptoms of poisoning than would be necessary in the case of the form of barium found in the plant, on the assumption that in the loco weed the barium is better protected from precipitation than are the barium salts when dissolved in water alone.

It seemed wise, then, in making this experiment, to start with amounts of barium salts as nearly as possible equivalent to those which were found in the proportion of loco weed that an animal would be likely to eat in his daily ration. It was planned to continue administering these small doses until symptoms of poisoning appeared. In case these symptoms did not appear it was planned to increase gradually the size of the dose until some positive effect was produced. Inasmuch as a number of animals were at the same time fed upon varying proportions of loco, it was possible, with somewhat large elements of error, to estimate how much barium salts these animals were receiving daily. The comparison of these animals with those which were receiving barium salts should give positive evidence in regard to the identity of loco poisoning and barium poisoning.

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<sup>1</sup> Crawford, A. C. Op. cit., pp. 75-76.

<sup>2</sup> Crawford, A. C. Op. cit., p. 75.

## GENERAL PLAN OF THE EXPERIMENTS.

As the result of the preceding work in feeding loco to cattle and horses, it was found that they would become locoed if fed daily from 6 to 16 pounds of the green material; therefore, we may conclude that an average daily ration of 12 pounds (6 kilograms) will produce loco poisoning. It was assumed that the loco was half water. On this basis the animal would get 3 kilograms (6.6 pounds) of dried loco daily. Using Crawford's figures that 12.44 per cent of the dried plant is ash and that there is in each gram of the ash 0.0026 gram of barium calculated as the sulphate, it would follow that the daily feed would contain 0.97032 gram of barium sulphate, or 1.0154 grams of barium chlorid.

It was on this basis that the experiments were started. When it was found as a matter of fact that the loco weeds actually used lost on the average one-third on drying, it followed that the daily dose would be 1.354 grams  $\text{BaCl}_2 + 2\text{H}_2\text{O}$ . As has been indicated before, the results of chemical analyses of the loco weeds which were fed during the experiments would not modify these figures materially. Theoretically, the daily administration of between 1 and 2 grams of barium chlorid should result in symptoms of loco poisoning. The possibility must be recognized, however, that many unknown factors in the problem may materially modify this theoretical determination.

## DETAILS OF THE EXPERIMENTS.

## FEEDING BARIUM SALTS TO CATTLE.

*Cow No. 92.*—The experiment with cow No. 92 was begun May 30, 1908. From this time to June 10 she received 1.13 grams of barium chlorid daily. This cow would not readily eat grain, so the barium chlorid was fed in solution on a small ration of alfalfa. On some days she may not have received the full dose, as it is possible that she did not eat all of the alfalfa. As a rule, however, most of the barium chlorid must have been taken. From June 11 until July 29 she was given 1.51 grams of barium chlorid daily. After July 29 the barium chlorid was given in bran, as it was found possible to feed her successfully with this form of grain. From August 21 until September 8 she received 10 grams of barium chlorid daily, fed in bran. From September 9 until the end of the experiment on September 26 she received 15 grams daily.

Figure 1 shows that at the beginning of the experiment this cow weighed 1,210 pounds, which increased during the course of the experiment to a maximum of 1,245 pounds on June 11. On July 2 she had fallen to 970 pounds, after which there was an increase, followed by a decrease, and her final weight at the end of the experiment was 970 pounds. During the course of the experiment there were no

symptoms of loco poisoning, with the possible exception of the loss in weight, and this may be accounted for by the birth of twin calves and the drain consequent upon nourishing them.

*Steer No. 46.*—From May 30 until June 10 steer No. 46 was given a daily dose of 1.13 grams of barium chlorid, the salt being fed most of the time in solution on alfalfa. From June 11 to July 21 the daily dose was 1.51 grams of barium chlorid on alfalfa. On two or three days during this time the steer ate none or only a part of its ration, but during most of the time it received nearly the full dose. From July 22 until August 11 it was fed

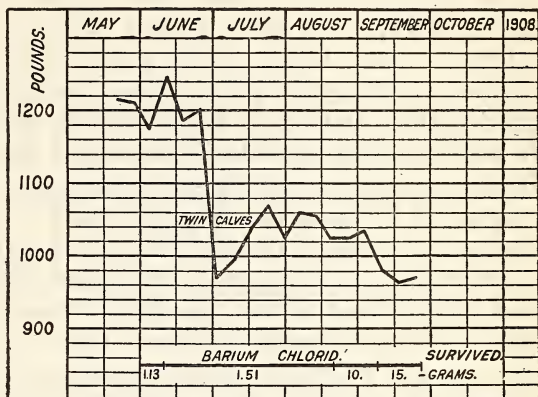


FIG. 1.—Curve of weight of cow No. 92.

5 grams of barium chlorid daily on alfalfa. From August 12 to 20 the same dose was given in bran. From August 21 to September 2 it was given 20 grams of barium chlorid daily in bran. From September 3 until September 8 it was given 40 grams daily in bran. From September 9 until September 15 it received 50 grams daily,

with the exception of September 11, when it received only 26 grams, and September 12 and 13, when the dose was omitted. From September 16 until the conclusion of the experiment on September 26 it received 60 grams daily of barium chlorid.

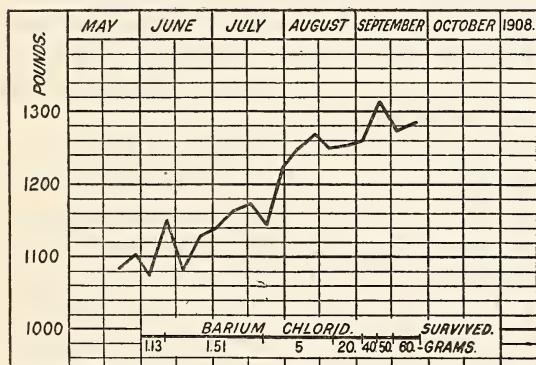


FIG. 2.—Curve of weight of steer No. 46.

be seen that at the beginning of the experiment this animal weighed 1,105 pounds. At the conclusion of the experiment it weighed 1,285 pounds and was in good condition in every way. No symptoms appeared showing any effect from the barium.

*Steer No. 10.*—As a result of being fed upon loco in preceding seasons steer No. 10 had been more or less affected by the poisoning,

although it was never considered to be distinctly locoed. It was in fair condition when the experiment commenced. From May 30 until June 10 it received 1.13 grams of barium chlorid daily in grain; from June 11 until June 29, 1.51 grams daily; from June 30 until July 6, 3 grams daily; from July 7 until July 13, 6 grams daily; from July 14 to 20, 9 grams daily; from July 21 until July 27, 12 grams daily; from July 28 until August 3, 15 grams daily; from August 4 until August 10, 20 grams daily; from August 11 until August 17, 25 grams daily; from August 18 until August 24, 30 grams daily. During the latter period it did not readily eat the bran in which the barium chlorid was mixed, since it evidently found so large a proportion of the salt distasteful. From August 25 until August 29 it was given 40 grams daily in bran, but because of its dislike it did not eat the full dose on any day.

Beginning with August 30 the barium chlorid was put in solution and administered to the animal as a drench in order to make sure that it received the full amount. From August 30 to September 1 the dose was 40 grams. From September 2 until September 8 it received 45 grams daily. On September 9 and 10 it received 50 grams. During the few days before this dose of 50 grams the animal had appeared to be in somewhat poor condition. On September 9 it seemed to be weak in its hind legs. In attempting to walk up an incline it had great difficulty because of this weakness. Otherwise it acted quite normally. On the morning of September 10 it was unable to use its hind legs sufficiently to get up. It appeared to have very little appetite. About noon it fell over upon its side and died with very little struggling. It should be noticed that the weakness of its hind legs was not due to any paralytic affection. Both the motor and sensory nerves seemed to be functional, only the animal did not have strength to use its limbs.

The autopsy was made immediately. A number of ulcers were found in the mucous membrane on the wall of the fourth stomach and the duodenum was somewhat inflamed. The brain was congested and small blood clots were found in the cavities. The heart was in systole. It is to be noted that this animal had been eating loco for three preceding seasons and the post-mortem appearances, especially the ulcers in the stomach, may have been due to loco poisoning rather than barium poisoning, although the barium was the immediate cause of death. There were no symptoms of barium poisoning preceding the death of the animal except the weakness of the hind legs spoken of and its generally poor appearance in the few days preceding its death.

The weight of this animal (fig. 3) when the barium treatment was commenced was 845 pounds. Near the conclusion of the experiment,

on September 3, it weighed 900 pounds. It will be seen that barium poisoning had very little effect in reducing its weight.

*Heifer No. 113.*—The experiment with heifer No. 113 was commenced on July 21. From July 21 until August 20 she was given 10 grams of barium chlorid daily. During most of this time the salt was fed on alfalfa, although for a few days it was fed in bran. From August 21 until September 5, 20 grams of barium chlorid were given daily in bran.

During several days of this period she ate only a part of the dose, the barium chlorid evidently being very distasteful. From September 6 until September 16, with the exception

of two days when no salt was available, the 20-gram dose was administered daily as a drench. From September 17 to 24 she received 25 grams daily as a drench.

At the beginning of the experiment this heifer weighed 415 pounds. Her weight (fig. 4) increased during the experiment, and at the conclusion she weighed 530 pounds; she showed no effects whatever of barium poisoning, unless the drop in weight after September 3 may

be attributed to its influence.

*Steer No. 114.*—The experiment with steer No. 114 was commenced on July 21. Until August 6 the dose was given on alfalfa, but after that until September 15 in bran. From July 21 until August 20 the animal re-

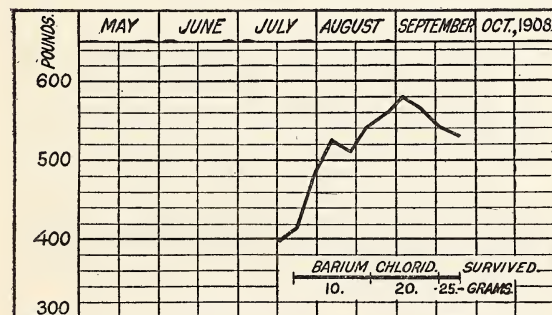


FIG. 4.—Curve of weight of heifer No. 113.

ceived 5 grams of barium chlorid daily. From August 21 until September 2 the dose was increased to 10 grams daily. From September 3 until September 8 the dose was 20 grams daily. From September 9 to 18 the dose was 25 grams daily. Inasmuch as the steer failed to eat all the grain because of its dislike of the taste of barium chlorid, from September 15 until September 18 the daily dose of 25 grams was given in solution as a drench.

During this time the animal showed no symptoms of barium poisoning. Its weight (fig. 5) from the beginning of the experiment had increased from 325 to 455 pounds. After receiving the dose of barium chlorid on the morning of September 18 it was driven into pasture with the other animals and appeared entirely well. During that day it was impossible to keep close watch upon the pasture, and the animal was not seen again until between 5 and 6 p. m., when it

was found dead. It had fallen and died apparently without a struggle.

An autopsy was made immediately. The heart was found in systole. The mucous membrane of the wall of the fourth

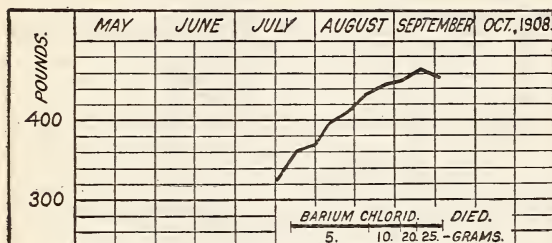


FIG. 5.—Curve of weight of steer No. 114.

stomach was found slightly inflamed. The only noticeable feature of the autopsy was a deeply inflamed condition of the small intestine, indicating very sharp irritation. This was rather striking, inasmuch as there had been no evidence of looseness of the bowels.

*Heifer No. 115.*—Five grams of barium acetate on alfalfa were fed daily to heifer No. 115 from July 21 to 30. From July 31 until August 20, with the exception of one day when the dose was omitted, she was given 5 grams daily in bran. From August 21 until September 2 she received 10 grams of barium acetate daily in bran. From September 3 until September 14 she was given 20 grams of barium acetate daily in bran. During the last few days of this

series of doses the heifer did not eat the bran readily because of her distaste for the barium acetate, so from this time until the completion of the experiment the barium acetate was given in solution as a drench. September 15 and 16 she received 20 grams daily, and from September 17 until the conclusion of the experiment on September 24 she received 25 grams daily.

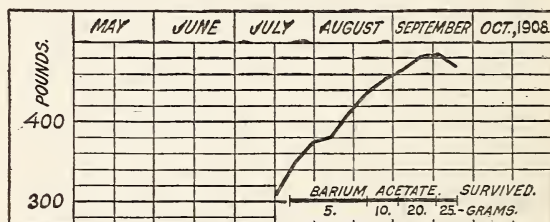


FIG. 6.—Curve of weight of heifer No. 115.

At the beginning of the experiment the heifer weighed 350 pounds, and at the end of the experiment (fig. 6), 470 pounds. The barium administered apparently had no effect upon the animal in any way,

either in reduction of weight or in the production of nervous symptoms.

*Steer No. 116.*—From July 21 until August 7 steer No. 116 received daily 10 grams of barium acetate on alfalfa. On August 8 the dose was omitted. Then the 10-gram dose was continued until August 20, being given during this period in bran. From August 21 until September 2 the dose was increased to 20 grams daily, in bran. From September 3 to September 8 the dose was 30 grams. From September 9 until September 16, with the exception of two days, September 12 and 13, the steer was given 35 grams, the salt being administered in a drench. On September 16 the dose of 35 grams was given about 8.30 a. m. The animal then left the corrals, went out into the pasture, and about 10.30 a. m. was found dead about half a mile from the corrals. It had died apparently without a struggle. There was no evidence that it had moved even a foot after falling to the ground.

An autopsy was made immediately. The mucous membrane lining the wall of the fourth stomach was in part inflamed, with ecchymotic spots at the pyloric end. The inner wall of the duodenum

was inflamed and the ileum was deeply inflamed. The mesenteric blood vessels in this case did not appear to be injected, as was true in many other cases.

This animal weighed at the beginning of the experiment 600 pounds (fig. 7). On September 10, a few days before its death, its weight was 690 pounds. It evidently had not lost weight and there had been no symptoms of poisoning. It had apparently been in entirely good health up to the morning of its death.

*Calf No. 109.*—Born June 29 of cow No. 92, calf No. 109 was one of a pair of twins. As both were in good condition it was considered that this was a good case for barium feeding, as he could easily be compared with his twin sister, No. 108. From July 22 until August 20, with the exception of one day, when the dose was omitted, he was given 1 gram of barium acetate daily in a drench. From August 21 to September 2 he was given 3 grams of barium acetate daily. From September 3 until September 8 the dose was 5 grams. From September 9 until September 15 he was given 8 grams. September 16, 17,

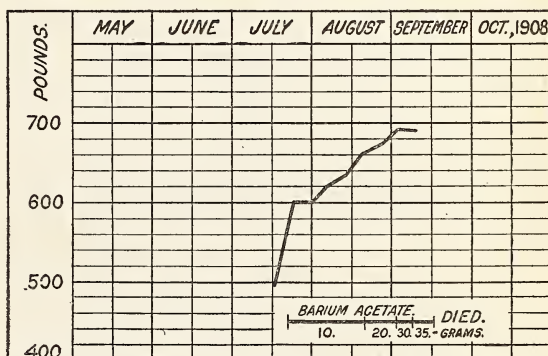


FIG. 7.—Curve of weight of steer No. 116.

and 18 he received 10 grams daily. During this time he had shown no symptoms of being affected by the poison. After the dose on the morning of September 18 he went into the pasture as usual and was not seen again until in the afternoon, between 5 and 6 o'clock, when he was found dead. He had evidently fallen and died without a struggle.

An autopsy was made on the morning of September 19. During the period of the feeding, the calf had gained weight continuously, its condition comparing very favorably with its twin. In the autopsy the heart was found in systole. The inner wall of the fourth stomach was slightly inflamed. The inner wall of the ileum was deeply inflamed in spots, although the inflammation was not general through the whole length of the small intestine. The brain was congested, and the mesenteric blood vessels were somewhat injected.

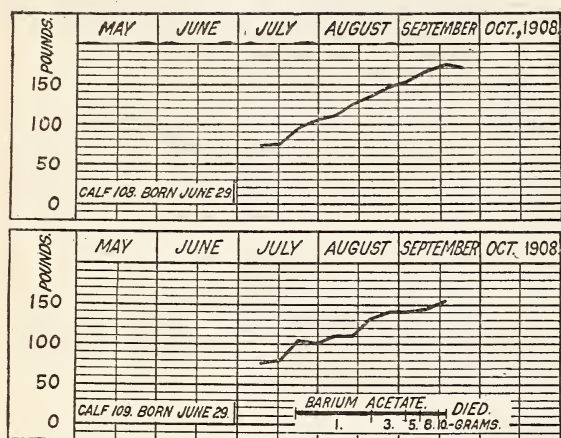


FIG. 8.—Curves of weights of calves Nos. 108 and 109.

Figure 8 shows the curve of weight of calf No. 109; also the curve of weight of No. 108 for purposes of comparison.

*Acute barium poisoning of Heifer No. 99.*—As will be brought out later, it appeared from the preceding experiments that 1 gram of the barium salt for about 18 pounds of weight proved to

be a fatal dose in the cases where the animals were receiving gradually increasing doses. It seemed wise to see what effect this amount of barium would have if given immediately to an animal without any preceding course of small doses.

Heifer No. 99 was a strong, healthy animal, thought to be a good subject for such an experiment. On September 20 she was given 22 grams of barium acetate as a drench. No effects were noticed. The next day she was given the same amount. She died about 5.30 p. m. Just before this time she was found lying on her belly, with her legs doubled up under her and her head thrown down on the ground to one side. She acted as though in pain, although she did not struggle. Passages from the bowels covered with mucus indicated an inflamed condition of the intestines. Death occurred a few minutes after she was found in this position. At about 2.30 p. m. she had been noticed

lying down not far from the location in which she died, but apparently she was all right at that time.

An autopsy was made on the morning of September 22. It was noticed that the superficial blood vessels of the heart were injected and the inner walls of both auricles and ventricles were deeply inflamed. The walls of the second and fourth stomachs were deeply inflamed, and the leaves of the third stomach were infiltrated with inflamed spots, nearly the whole surface being reddened. The whole small intestine was very deeply inflamed and the cæcum slightly so. It was evident that the barium poisoning had produced profound inflammation of the alimentary canal. Apparently death had resulted from the effect upon the circulatory and respiratory systems. Figure 9 shows the curve of weight of the animal.

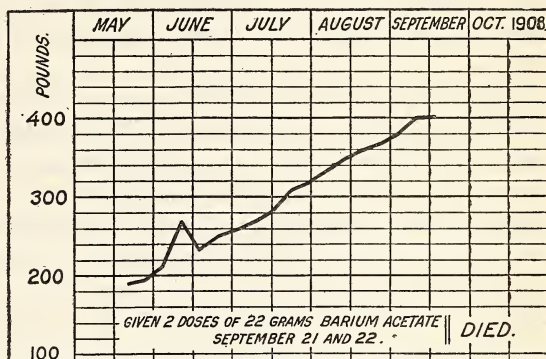


FIG. 9.—Curve of weight of heifer No. 99.

#### FEEDING BARIUM SALTS TO HORSES.

*Horse No. 62.*—Horse No. 62 had been fed loco in preceding seasons, but had never shown signs of being seriously affected by poisoning.

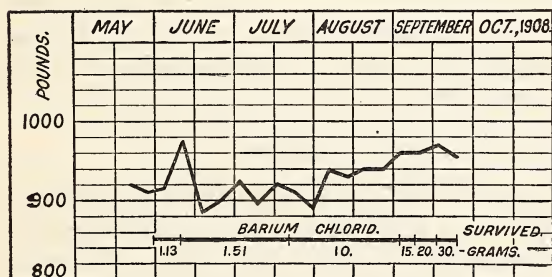


FIG. 10.—Curve of weight of horse No. 62.

From May 30 to September 24, he was given barium chlorid in grain. From May 30 to June 10 he was fed 1.13 grams of barium chlorid daily; from June 11 to July 21, 1.51 grams; from July 22 until September 2, 10 grams; from September 3 until September 8, 15 grams; from September 9 until September 16, except on September 12 and 13, 20 grams; and from September 17 until the conclusion of the experiment on September 24 he received 30 grams of barium chlorid daily.

At the beginning of the experiment this horse weighed 910 pounds, and at the conclusion of the experiment 955 pounds (fig. 10).

He was in remarkably good condition during the whole summer, his coat being smooth and silky—in fact there was no better looking horse on the ranch. There was no symptom of any effect from the doses given.

*Horse No. 61.*—Horse No. 61 had never been fed on loco at the time the experiment was commenced. Although he was an old horse, he was in thoroughly good condition and was considered a good animal for such an experiment. From July 22 until September 2 he he was fed 10 grams of barium acetate daily. This was fed most of the time in bran or chop, inasmuch as he ate grain very freely. The dose was omitted during this period on only two days. From September 3 until September 8 he was fed 15 grams of barium acetate daily. From September 9 until September 16 the dose was increased to 20 grams. From September 17 until the conclusion of the experiment on September 24 he was fed 35 grams of barium acetate daily.

At the beginning of the experiment this horse weighed 825 pounds (fig. 11). At the conclusion of the experiment he weighed 830

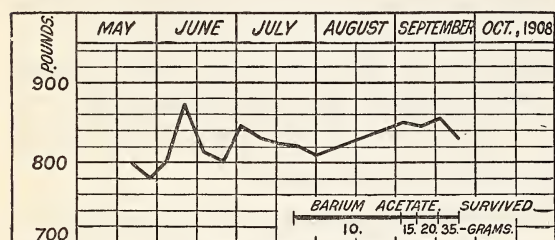


FIG. 11.—Curve of weight of horse No. 61.

pounds. The weight had continued pretty nearly uniform during this time. Inasmuch as he was an old animal in good condition when the experiment started, under normal conditions we could expect

his weight to remain nearly constant. There were no symptoms whatever of poisoning and the animal at the conclusion of the experiment was in fine condition.

#### EXPERIMENTAL WORK ON RABBITS.

It seemed best, in connection with the barium feeding, to make a few experiments on rabbits as a check on the work with other animals. Loco (*Aragallus lamberti*) was fed to six rabbits, Nos. 3, 7, 8, 10, 11, and 12. The plan was to make the full feeding of loco, but as the experiment progressed it was found that the rabbits did not have sufficient appetite for the weed to eat any very large amount, although very little other food was supplied them. They were fed through periods varying from a few days to something over a month.

Rabbit No. 3 was fed from August 29 to September 28, the quantity received varying from 100 to 200 grams daily. It showed no symptoms of poisoning, and the fact that its weight remained constant showed that it did not suffer from starvation.

Rabbit No. 7 was fed for three days, when it died. It was troubled with diarrhea and its death was evidently caused by something else than loco.

Rabbit No. 8 was fed for eight days but refused to eat much of the loco, and the experiment had to be given up for fear of starvation.

Rabbit No. 10 was fed 100 to 200 grams daily from September 4 until September 28. It showed no symptoms of loco poisoning, but exhibited a marked loss in weight, falling from about 2,300 to 1,650 grams.

Rabbit No. 11 was fed loco from September 1 until September 28, receiving from 100 to 200 grams daily. It decreased in weight from about 1,950 to 1,750 grams, but showed no starvation symptoms. No symptoms of loco poisoning appeared.

Rabbit No. 12 died after about four days' feeding, of acute inflammation of the cæcum. Death was evidently due to an acute infection and was not connected in any way with loco.

The feeding of loco was entirely inconclusive, and it is to be presumed that none of the animals received enough of the poison to produce the characteristic symptoms.

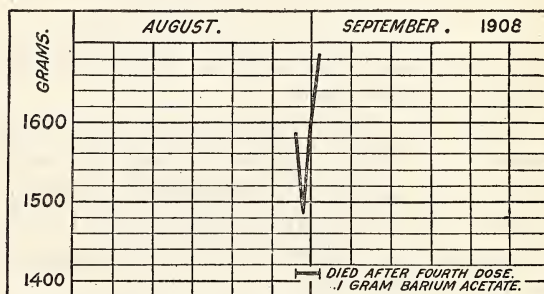


FIG. 12.—Curve of weight of rabbit No. 1.

Barium salts were fed to five rabbits, Nos. 1, 2, 4, 5, and 9. The salts were administered in solution through a stomach tube, and No. 6 was given an equal dose of water daily as a control.

Rabbit No. 1 received four daily doses of 0.1 gram of barium acetate. About 15 minutes after receiving the fourth dose, the animal was found lying with its hind legs motionless and moving its fore legs but very little. It gasped, its respirations and heart action were very rapid, and in about half an hour after the administration of the dose it was dead. The condition of the hind legs was not strictly that of paralysis, but rather that of weakness, as it had possibilities of both sensation and motion. An autopsy was made immediately. The mesenteric blood vessels were found very much congested. No other characteristic symptoms of poisoning appeared. During the feeding of barium this rabbit increased in weight (see fig. 12), so that there was no question of starvation.

Rabbit No. 2 received six daily doses of 0.1 gram of barium acetate, but showed no symptoms of poisoning until the sixth dose. This was administered about 10.30 a. m. Immediately after, the rab-

bit fell on its side with apparent symptoms of partial paralysis, the hind limbs being more affected than the fore limbs. On stimulation, however, it was found that the limbs were not paralyzed. It gasped, had violent convulsions, and died at 10.40. In the autopsy the walls of the stomach were found inflamed. The inner wall of the duode-

num was inflamed, the mesenteric blood vessels were congested, and the spinal cord and brain were congested. There were blood clots in the epidural space of the spinal column. Rabbit No. 2 gained rapidly in weight during the period of

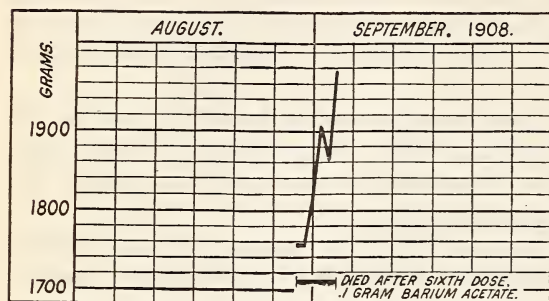


FIG. 13.—Curve of weight of rabbit No. 2.

the administration of barium. Figure 13 shows the curve of weight during the experiment.

Rabbit No. 4 was fed barium acetate from August 29 until September 13. From August 29 until September 8 it received daily doses of 0.05 gram; after that 0.075 gram daily. During this period it gained in weight from 1,566 grams to 1,846 grams (fig. 14) and apparently was entirely healthy, showing no symptoms of poisoning.

A few minutes after receiving its dose of 0.075 gram on September 13 it was stretched out on its belly, gasping. It did not appear extremely weak and when startled would move about readily. It apparently gradually recovered and was put in the pen. The dose was given between 10 and 10.15

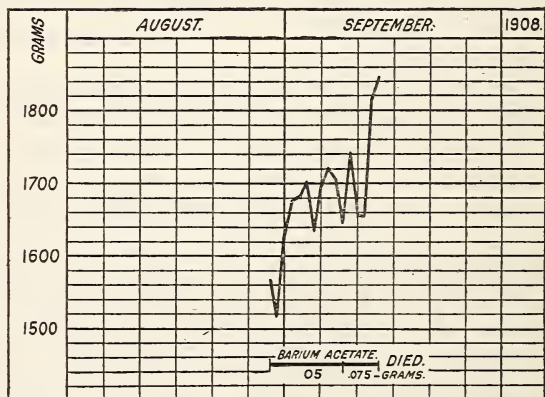


FIG. 14.—Curve of weight of rabbit No. 4.

a. m.; by 10.45 it was found on its side in the pen with its hind legs motionless. Respiration was about 120, the temperature was 103.6, and the heartbeats were extremely rapid. At 11.35 it was able to kick with its hind legs, showing that there was nothing like complete paralysis. It lay upon its belly with its head erect. Respiration at this time was 120. At 12 o'clock on being stimulated it

brought up its hind legs under it, sat up, and jumped off, but moved somewhat awkwardly and made only two or three jumps. As it sat up it acted a little sleepy. It was left at 12.15 and was found dead at 12.30. In the autopsy a few blood clots were found in the lungs. That some water had passed into the larynx in the process of dosing seemed probable and perhaps the effect of the barium poisoning was thus complicated. The mesenteric blood vessels were injected, the cardiac portion of the stomach was inflamed in its inner wall, and there were patches of extravasated blood. The inner walls of the small intestines were inflamed. It was noticed in this animal, too, that the cystic veins were injected.

Rabbit No. 5 was fed barium salts from August 29 to September 28. From August 29 to September 8 it received 0.05 gram of barium acetate daily; from September 9 to September 17, 0.075 gram daily; from September 18 until September 26, 0.1 gram daily. On September 27 it was given 0.12 gram and on September 28, 0.15 gram. During this period it increased in weight from 1,676 to 2,362 grams (fig.

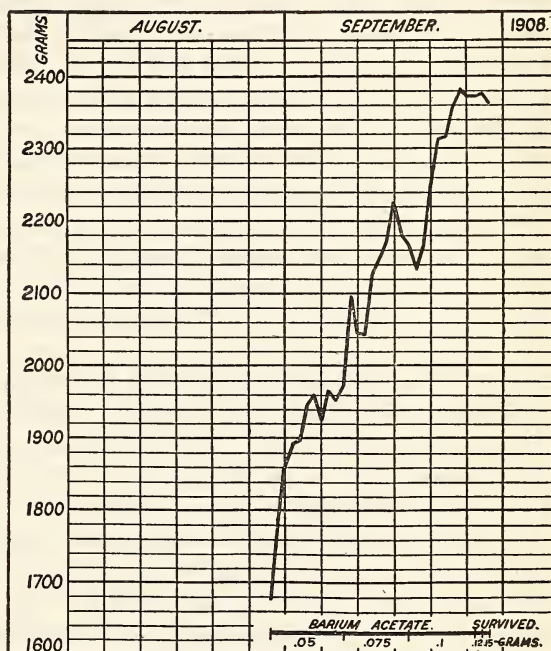


FIG. 15.—Curve of weight of rabbit No. 5.

15) and showed no symptoms of any effect from the barium.

Rabbit No. 9 weighed 2,076 grams. It was given two doses of 0.2 gram of barium acetate on September 1 and 2. After the second dose, which was given in the morning, it appeared to be all right, but at 6 p. m. it was found lying on its side with its hind legs extended and motionless. The heartbeats were from 160 to 190 per minute. It was visited again at 6.45. While it was being watched it developed convulsive movements of the head, forelegs, and tail. It was seen again at 7.15; the convulsive movements were continued. It died during the night. In the autopsy the heart was found in systole, the mesenteric blood vessels were congested, inflamed areas were

found in the walls of the stomach, and slight evidence of inflammation in the walls of the duodenum. The brain was considerably congested and considerable blood was found in the epidural space of the spinal cord. This was evidently a case of acute barium poisoning.

#### DISCUSSION OF THE EXPERIMENTAL FEEDING OF BARIUM.

##### RELATION OF LETHAL DOSE TO WEIGHT.

In connection with the question of the cumulative effect of barium poisoning it is of interest to note how much of the barium salts was taken before the effects of poisoning appeared. In the rabbits which died this varied from 0.4 gram to 0.925 gram, or in terms of ratio to weight from 1:1,816 to 1:5,145. With the exception of No. 4, which was fed barium acetate from August 29 to September 13, all the rabbits died after receiving from 4 to 6 daily doses. Rabbit No. 5 received a total of 2.445 grams of barium acetate in 30 daily doses, receiving 1/1,031 of its weight without any effect whatever.

In the cattle that succumbed to the poison the total amount of barium salts received varied from 44 grams in No. 99 to 1,437 grams in No. 10. It should be noted in this connection that No. 10 was the only full-grown animal in the number and that the amount which No. 99 received was given in two daily doses. The ratio of the total amount of barium salts received to the weight in the fatal cases was as follows: No. 10, 1:313; No. 114, 1:367; No. 116, 1:316; No. 99, 1:4,545; No. 109, 1:344. If we eliminate the acute case, No. 99, there would seem to be a fair degree of uniformity in these ratios, but this uniformity disappears when we compare them with the ratio of those animals that suffered no harm from the administration of barium salts: No. 46, 1:327; No. 92, 1:1,091; No. 115, 1:286; No. 113, 1:258; No. 61, 1:642; and No. 62, 1:768. As No. 115 received 1/286 of its weight and No. 113 received 1/258, more than in any of the fatal cases, it would appear that no definite limit can be assigned as the total which an animal may receive, and that, presumably, small doses might be taken almost indefinitely without any bad effects.

##### LETHAL DOSE.

Crawford has discussed the literature of barium poisoning in rabbits and has made a considerable number of experiments bearing upon the question of the lethal dose in these animals; so it is not necessary to discuss the matter here, as the additional experiments were few in number. Doses of 0.075 to 0.1 gram ordinarily proved fatal. The ratio of dose to weight which finally proved fatal varied from 1:1,029 to 1:22,400, while No. 5 received 1/16,800 of its weight with no ill effects.

These results correspond fairly well to those reported by Crawford and preceding authors. A wide limit of variation is evident. It would appear that doses above 0.5 gram in rabbits of average size may give fatal results. Generally speaking, rabbits are much more susceptible to barium poisoning than are horses and cattle.

Very little seems to have been published in regard to the lethal dose of barium for horses and cattle given per os. Huzard and Biron are quoted by Christison<sup>1</sup> as having killed a horse in 15 days by daily doses of 8 grams of  $\text{BaCl}_2$ , with no symptoms previous to death. Dieckerhoff<sup>2</sup> states that a dose of 8 to 12 grams of  $\text{BaCl}_2$  is dangerous to a horse and that a 2-year-old heifer received 40 grams with no effect. Reynolds<sup>3</sup> killed a horse with 15 grams of  $\text{BaCl}_2$ .

Granting that toleration is not acquired (and these experiments show little evidence of it), the lethal dose for horses and cattle appears, in these cases, to be considerably higher than the previously recorded results would lead one to expect. Horse No. 61 received 35 grams and horse No. 62 received 30 grams with no ill effects. No. 61 averaged about 850 pounds in weight and No. 62 about 950 pounds. One received  $1/12,143$  of its weight and the other  $1/15,833$  of its weight with no bad results; this is three times the dose which Dieckerhoff considers dangerous. The actual lethal dose for horses was not determined.

On the basis of a 1,000-pound animal it would appear from these results that the lethal dose for cattle is something over 50 grams. The smallest dose which produced death in the case of cattle was at the rate of 55 grams per 1,000 pounds of weight. Even this did not in all cases produce evidences of poisoning, for No. 115 received 62.5 grams per 1,000 pounds of weight without any symptoms of poisoning. In general it can be said, however, that the danger limit per 1,000 pounds of weight is reached when the dose exceeds about 50 grams. With cattle the ratio of fatal dose varied from 1:6,500 to 1:9,000, averaging 1:8,232, or, in other words, 1 gram to about 18 pounds of weight proved fatal when repeated two or three times. In two cases only did cattle receive such large doses and live. No. 115 received for 8 days a dose equal to  $1/8,000$  of its weight without any bad results. No. 109 received  $1/8,125$  of its weight for 7 days, and only succumbed after three doses of  $1/6,500$  of its weight. The experiment would seem to indicate that the danger line lies in a dose of about  $1/8,000$  of the animal's weight, but with considerable possibility of variation, due in part, probably, to the individual peculiarities of the animal. It may succumb to a dose of  $1/9,000$ ,

<sup>1</sup> Christison, R. *Treatise on Poisons*. Edinburgh, 1845, p. 578.

<sup>2</sup> Dieckerhoff, W. *Ueber die Wirkung des Chlorbaryum bei Pferden, Rindern, und Schafen*. *Berliner Thierärztliche Wochenschrift*, 1895, p. 265.

<sup>3</sup> Reynolds, M. H. *A Study of Certain Cathartics*. *Fifteenth Annual Report, Minnesota Agricultural Experiment Station*, 1907, pp. xxi-xlii.

or it may endure a dose of 1/6,500 or of 1/7,000 of its weight. There appears to be no appreciable difference in the toxic effect of the two salts, barium chlorid and barium acetate.

It should be noted that the animals treated with barium salts were watered at the creek and thus received a considerable amount of sulphates, which may have had some effect in neutralizing the barium. As has already been remarked, the amount of sulphates in the creek water would make an equivalent of about 40 grams daily of magnesium sulphate for the average quantity of water consumed by a 1,000-pound animal. These sulphates would theoretically neutralize an equivalent amount of a barium salt. The animals treated were brought into the corrals at night and the barium salt was given to them in the morning, or, in the case of larger doses, both at night and in the morning. After being dosed they were driven into the pasture, where they immediately went to feeding and did not ordinarily go to water for some time. This gave the barium a chance to produce an effect before coming in contact with the sulphates. The animals were not regular in their habits of drinking, however, and it is very possible that in some cases the lack of effect from small doses may have been due in part to the sulphates of the drinking water. The possibility should be recognized that the high lethal dose may be accounted for by the antidoting effect of the water used. It was not practicable, with the experimental material available, to feed barium to animals watered on sulphate-free water.

While there is this possibility of an effect of barium neutralization by the drinking water, it is the impression of the writer that in the experimental animals as a whole the toxicity of the barium salts was modified very little. This seems the more probable when it is remembered that the lethal dose for the rabbits at Hugo corresponded very well with the results recorded by others. The water used by the rabbits contained, per million, 169.17 parts  $\text{SO}_4$ , while the creek water used by the larger animals contained 637.4 parts  $\text{SO}_4$ .

#### CUMULATIVE EFFECT.

There is a certain amount of cumulative effect.

No. 10 succumbed after 7 doses of 45 grams each and one dose of 50 grams.

No. 114 received 25 grams daily for something over four days. It is impossible to tell just how much the steer took from September 9 to September 14.

No. 116 received 35 grams daily for eight days before succumbing.

No. 109 received 10 grams for three days.

In all these cases the barium was fed in gradually increasing amounts, and it had been found, as already stated, that the lethal dose

was in the neighborhood of 1 gram to 8,232 grams of weight. This amount was given to No. 99 and it succumbed after the second dose.

While there is a cumulative effect in large doses, small doses seem to have no similar result. Possibly the poison is excreted with sufficient rapidity to prevent any toxic effect from small doses.

#### TOLERATION.

There is little evidence of toleration or acquired immunity to the effects of the poison. No. 10, which received doses of barium for nearly four months, succumbed to the same dose relatively to its weight as that which killed No. 99 in two doses. In all cases the length of time during which the doses were repeated seemed to have little effect in modifying the lethal dose.

#### SYMPTOMS.

Small doses had no effect upon the appetite and gave no loss of weight. During the period when the doses approached the toxic limit there seemed to be a small loss of weight in most cases. This loss of weight was especially pronounced in steer No. 10. With rabbits death was preceded by a period of weakness, when the hind legs seemed to be especially affected, accompanied by rapid respiration, rapid heartbeats, and convulsions. To most of the cattle death came very suddenly, with few pronounced symptoms. In steer No. 10 the symptoms were more noticeable than in any other case; its previous loco poisoning may have had something to do with this condition. Generally speaking, with the cattle small doses produced no symptoms, even when continued for a long time. When the dose which was to prove fatal was reached, symptoms of weakness and pain might be developed, but in most cases death seems to have come with no previous symptoms. Apparently the animal fell in its tracks and died without much movement.

#### POST-MORTEM APPEARANCES.

The post-mortem appearances agreed in general with those noticed by previous writers on the subject of barium poisoning. The heart was generally in systole, the left ventricle being almost always contracted and empty. The mesenteric blood vessels were injected and, generally, the central nervous system was congested. The inner walls of the stomach and small intestines were more or less inflamed. The inflammation in the stomach was more marked in the pyloric end, and in the intestines inflammation was more distinct in the duodenum and cæcum. The large intestines were not affected. The extent of the inflammation varied with the acuteness of the poisonous effect, but was always marked. No ulcers were noticed except in No. 10, and in this animal they may have been due to previous loco poisoning.

**COMPARISON OF LOCO POISONING WITH BARIUM POISONING.**

The symptoms of loco poisoning have been summarized by the writer in a previous publication and may be briefly stated as follows: The first pronounced symptom is in the gait, which is stiff, with more or less evidence of partial paralysis. There is a lack of muscular coordination, which produces "high stepping," rearing and jumping, and stumbling. In drinking, the mouth moves in a peculiar way, something like in eating, a movement which is more easily recognized than described. The animal is either dull and dejected or in constant motion, as the result of a disorganized nervous system. It gradually loses flesh, its coat becomes rough, its eyes staring, it becomes profoundly anæmic, and eventually it dies of starvation. Ulcers are found on the inner walls of the stomach of horses and of the fourth stomach of cattle and sheep. The brain and spinal cord are hyperæmic and a mass of serous exudate more or less coagulated is formed in the epidural space of the spinal canal. Abortion is common in locoed animals. It should be noticed, too, that these symptoms come on gradually as a result of prolonged feeding.

In the animals subjected to prolonged barium feeding no peculiarities in walking were noticed, such as the stiff gait and high stepping, nor any evidence of muscular incoordination. With the exception of steer No. 10, all the animals used their limbs in the normal manner. Weakness in the hind limbs of the rabbits at the time when the toxic dose was reached, as in steer No. 10, was not comparable with the paralytic effects in the locoed animals. There was no effect upon the muscles of the mouth. The animals showed no dullness and no unusual nervous symptoms. They gained in weight during the prolonged feeding of small doses or, as in the cases of the two horses which were in fine condition at the beginning of the experiment, held their own with no loss. There was no loss in weight until the dose was increased to the limit which produced acute symptoms. The barium-fed animals did not become anæmic. No ulcers were found in the stomach. No serous exudate collected in the spinal canal. The congested condition of the central nervous system, however, may be compared with the hyperæmia of the brain and cord in the locoes. Barium is said to produce abortion and while our experiments are not conclusive on this point we had one or two cases of abortion in rabbits which may have been caused by the barium. The noticeable thing in the comparison is that the general nervous symptoms and emaciation, which are usually considered characteristic of chronic loco poisoning and are very easily recognized by one familiar with cattle and horses in loco-infested regions, were entirely absent.

There was not the slightest evidence that the barium-fed animals became locoed. This becomes strikingly evident when barium-fed

animals are compared with those to which loco was fed. One gram of the loco weeds which were fed in Hugo during the season of 1908, according to the analyses of the Bureau of Chemistry, contained on an average an equivalent of nearly 0.0002 gram of barium salts. Knowing the amount of loco fed daily, it is easy on the basis of these analyses to estimate approximately the amount of barium salts received by the animals to which loco was fed in the corrals. In the case of the corral-fed animals Table II shows the average daily feed of loco, the approximate amount of barium salts contained in this ration, and the length of time which elapsed before symptoms of loco poisoning developed.

TABLE II.—*Average daily ration of loco, the approximate amount of barium salts contained therein, and the length of time required to develop loco poisoning.*

| Serial No. of animal. | Daily ration. | Barium salts. | Time required to develop poisoning. |
|-----------------------|---------------|---------------|-------------------------------------|
|                       | Pounds.       | Grams.        | Days.                               |
| Cattle:               |               |               |                                     |
| 35.....               | 5             | 0.5           | 77                                  |
| 6.....                | 10            | 1.0           | 70                                  |
| 84.....               | 10            | 1.0           | 101                                 |
| 68.....               | 7             | .7            | 75                                  |
| Horses:               |               |               |                                     |
| 55.....               | 8             | .8            | 71                                  |
| 57.....               | 8             | .8            | 33                                  |
| 70.....               | 8             | .8            | 75                                  |

In like manner, for purposes of comparison, Table III shows the daily doses of barium salts fed to the animals and the length of time the experiment was continued during which no symptoms of loco poisoning developed.

TABLE III.—*Daily doses of barium salts fed to animals and length of time fed with no resulting symptoms of loco poisoning.*

| Serial No. of animal. | Barium salts. | Length of time fed. |
|-----------------------|---------------|---------------------|
|                       | Grams.        | Days.               |
| Cattle:               |               |                     |
| 92.....               | 1.13 to 15    | 119                 |
| 46.....               | 1.5 to 60     | 119                 |
| 10.....               | 1.5 to 50     | 104                 |
| 113.....              | 10.0 to 25    | 68                  |
| 114.....              | 5.0 to 25     | 59                  |
| 115.....              | 5.0 to 25     | 68                  |
| 116.....              | 10.0 to 35    | 58                  |
| Horses:               |               |                     |
| 61.....               | 10.0 to 35    | 72                  |
| 62.....               | 1.15 to 30    | 123                 |

In comparing Tables II and III it will be noticed that for all the animals which were fed weighed rations of loco in the corrals, the amount of barium salts received in the loco varied from 0.5 to 1 gram daily and that these animals showed distinct signs of loco poisoning at periods varying from 33 to 101 days. On the other

hand, the animals receiving doses of barium received amounts varying from 1.13 to 60 grams daily. In all cases the small dose was continued for a considerable period of time, until it was evident that no symptoms would develop. These animals were fed for periods varying from 58 to 123 days. The periods during which barium salts were fed are therefore seen to be rather longer than those during which loco was fed to the animals in the corrals. The total amounts of barium received in the cases of the barium-fed animals were, in the long run, very much greater than in the cases of the loco-fed animals. If only large doses of barium had been fed, it might be said that the cases of poisoning were all cases of acute poisoning; but the smaller doses were continued for a long period of time, and large doses were administered only when it appeared certain that no symptoms of so-called loco poisoning would develop and when it seemed best to determine the toxic limit for a given animal. If barium alone would produce symptoms of so-called loco poisoning, some one of the long list of animals which were fed during the summer should have developed the characteristic symptoms of this disease. In the light of these facts the failure in the experiments of using sulphates as antidotes for loco poisoning would seem to be partially explained. If barium were the cause of the poisoning, it would seem probable that the administration of sulphates would at least produce some mitigating effect, even had it not served as a perfect antidote. The fact that the animals which received these sulphates were locoed fully as quickly as they would have been without the administration of the antidote would lead one to suspect that the true cause of the symptoms of loco poisoning was something other than barium. Reference may be made in this connection to C. D. Howard's paper on "Occurrence of Barium in the Ohio Valley Brines and Its Relation to Stock Raising."<sup>1</sup> There seems to be no doubt that he rightly ascribes the deaths of the animals to barium poisoning, and that it is probable that deaths of other animals have been caused in this region in the same way. Yet this is a part of the country where symptoms like those seen in locoed animals are unknown.

While these results seem to indicate clearly that barium will not produce the symptoms of so-called loco poisoning, it should be noted, perhaps, that it does not by any means follow that barium may not be a factor in the production of this disease. It would appear that there are in the loco plants certain properties or combinations of properties as yet undiscovered which will produce the series of symptoms known as loco poisoning.

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<sup>1</sup> Bulletin 103, West Virginia Agricultural Experiment Station, 1906.

## SUMMARY.

(1) Granted that barium is the cause of loco poisoning, sulphates form a logical antidote.

(2) Experimental administration of magnesium sulphate and sulphuric acid in amounts much greater than those necessary to neutralize barium in the loco eaten had no antidotal effect.

(3) Experimental feeding of barium salts produced symptoms which corresponded in general to those recorded in literature in acute poisoning, but without marked cumulative effect or acquired toleration, while the lethal dose was higher than that noticed by most observers. The post-mortem appearances corresponded to those recorded in acute poisoning.

(4) The symptoms and pathology of barium poisoning differed in a marked degree from those noted in loco poisoning. Typical loco poisoning is not produced by barium feeding alone.



## II.—LABORATORY STUDIES ON THE RELATION OF BARIUM TO THE LOCO-WEED DISEASE.

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### INTRODUCTION.

Dr. A. C. Crawford<sup>1</sup> has reported the presence of barium in loco plants from Colorado and has published a chemical and pharmacological study on which he bases the conclusion that barium is a cause of the loco-weed disease. A conclusion of so much practical interest and importance as this would seem to demand that further study be given to the action of barium in the loco-weed disease. It gives rise to a series of problems connected with the possibility that plants may, under certain conditions, derive harmful constituents from the soil. One of these problems is to determine whether barium is also taken up by other plants growing on the same barium-containing soils as the loco plants or whether the loco plants possess a specific selective preference for barium as a result of which they alone, of all the plants growing under the same condition in these regions, absorb enough to become toxic. Another problem is to investigate whether plants from other parts of the United States where barium occurs as a mineral also contain this metal and if so whether they also are toxic. The form in which barium occurs in these plants and in their ash is also a question of considerable interest. Still another problem has been investigated by the Bureau of Soils, the results of which show that barium is widely distributed in soils.<sup>2</sup> Some of these problems have been studied and the results are presented in this paper.

### OCCURRENCE OF BARIUM IN VARIOUS PLANTS FROM COLORADO, WYOMING, AND ARIZONA.

The first step in this investigation was a search for barium in different species of plants from the regions where the loco-weed disease prevails.

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<sup>1</sup> Crawford, A. C. Barium, a Cause of the Loco-Weed Disease. Bulletin 129, Bureau of Plant Industry, U. S. Dept. of Agriculture, 1908.

<sup>2</sup> Failyer, G. H. Barium in Soils. Bulletin 72, Bureau of Soils, U. S. Dept. of Agriculture, 1910.

The determinations of barium were made in the manner described by Hillebrand.<sup>1</sup> In most cases, as a matter of precaution, the barium sulphate finally obtained was again fused and treated with oxalate in the usual way, in order to remove traces of any calcium that may have contaminated it. In many cases the precipitates were examined with the spectroscope for contamination. They never carried more than a trace of calcium. The results are given in Table IV.

TABLE IV.—*Barium content of various plants from Colorado, Wyoming, and Arizona.*

| Analysis No. | Plant, collection number, and locality.                                               | Moisture in air-dried plant. | In dried plant. |                  |               | In ash.          |               |
|--------------|---------------------------------------------------------------------------------------|------------------------------|-----------------|------------------|---------------|------------------|---------------|
|              |                                                                                       |                              | Ash.            | Barium sulphate. | Calcium oxid. | Barium sulphate. | Calcium oxid. |
|              |                                                                                       | Per cent.                    | Per cent.       | Per cent.        | Per cent.     | Per cent.        | Per cent.     |
| 1            | Alfalfa (M. 90, Hugo, Colo.).....                                                     | 9.32                         | 8.55            | 0.014            | .....         | 0.150            | .....         |
| 2            | Alfalfa (M. 9.65, Limon, Colo.)....                                                   | 6.34                         | 9.14            | .....            | .....         | .....            | .....         |
| 3            | Alfalfa (M. 9.43, Hugo, Colo.).....                                                   | 6.96                         | 6.47            | .010             | .....         | .159             | .....         |
| 4            | <i>Astragalus nitidus</i> Dougl. (Wood-land Park, Colo.).....                         | 8.53                         | 7.21            | .003             | 1.47          | .041             | 19.52         |
| 5            | <i>A. mollissimus</i> Torr. (M. 9.95, Hugo, Colo.).....                               | 8.50                         | 9.94            | .043             | .....         | .423             | .....         |
| 6            | <i>A. simplicifolius</i> Gray (M. 9.4, Buckman, Wyo.).....                            | 6.14                         | 10.03           | .042             | .....         | .198             | .....         |
| 7            | <i>A. mollissimus</i> Torr. (M. 9.195, Hugo, Colo.).....                              | 5.58                         | 16.60           | .032             | .....         | .168             | .....         |
| 8            | <i>A. thurberi</i> Gray (Tm. 3, Calabazos, Ariz.).....                                | 6.69                         | 17.48           | .029             | .....         | .088             | .....         |
| 9            | <i>A. mollissimus</i> Torr. (M. 9.195, Hugo, Colo.).....                              | 5.58                         | 24.74           | .022             | .....         | .088             | .....         |
| 10           | <i>Aragallus lamberti</i> (Pursh) Greene (M. 8.96, Hugo, Colo.).....                  | 5.79                         | 28.90           | .029             | .....         | .100             | .....         |
| 11           | <i>A. lamberti</i> (Pursh) Greene (M. 9.68, Limon, Colo.).....                        | 5.91                         | 6.09            | .016             | .....         | .273             | .....         |
| 12           | <i>Lupinus pusillus</i> Pursh (M. 9.27, Mount Carbon, Colo.).....                     | 7.84                         | 7.71            | .009             | .....         | .120             | .....         |
| 13           | <i>L. sericeus</i> Pursh (M. 9.38, Hugo, Colo.).....                                  | 7.56                         | 8.18            | .....            | .....         | .....            | .....         |
| 14           | <i>L. pusillus</i> Pursh (M. 9.71, Limon, Colo.).....                                 | 7.20                         | 7.47            | .003             | .85           | .034             | 11.36         |
| 15           | <i>L. parviflorus</i> Nutt. (M. 9.56, Mount Carbon, Colo.).....                       | 7.14                         | 17.82           | .023             | .....         | .131             | .....         |
| 16           | <i>Buchloe dactyloides</i> (Nutt.) Engelm., buffalo grass (M. 9.46, Hugo, Colo.)..... | 9.37                         | 17.81           | .023             | .....         | .160             | .....         |
| 17           | Barley (M. 9.49, Hugo, Colo.).....                                                    | 9.80                         | 23.01           | .006             | .....         | .027             | .....         |
| 18           | Millet (M. 9.41, Hugo, Colo.).....                                                    | 9.80                         | 16.31           | .019             | .....         | .114             | .....         |
| 19           | <i>Andropogon scoparius</i> Michx. (grass; M. 9.47, Hugo, Colo.).....                 | 5.74                         | 16.26           | .021             | .....         | .130             | .....         |
| 20           | Beet (M. 9.204, Hugo, Colo.).....                                                     | ( <sup>1</sup> )             | 10.60           | .017             | 3.90          | .168             | 36.87         |
| 21           | <i>Yucca glauca</i> Nutt. (M. 9.208, Hugo, Colo.).....                                | ( <sup>1</sup> )             | 6.85            | .005             | .....         | .069             | .....         |
| 22           | <i>Calamovilfa longifolia</i> (Hook.) Hack. (M. 9.115, Hugo, Colo.).....              | ( <sup>1</sup> )             | 5.51            | 0                | 1.95          | 0                | 35.40         |
| 23           | <i>Artemisia filifolia</i> Torr. (M. 9.119, Hugo, Colo.).....                         | ( <sup>1</sup> )             | 14.45           | .015             | .....         | .104             | .....         |
| 24           | <i>Ipomoea leptophylla</i> Torr. (M. 9.206, Hugo, Colo.).....                         | ( <sup>1</sup> )             | 10.07           | .008             | .....         | .079             | .....         |
|              |                                                                                       |                              | 14.53           | .006             | .....         | .043             | .....         |
|              |                                                                                       |                              | 1.11            | 0                | .95           | 0                | 8.60          |
|              |                                                                                       |                              | 5               | 0                | .....         | .....            | .....         |
|              |                                                                                       |                              | 5.5             | 0                | .....         | .....            | .....         |
|              |                                                                                       |                              | 4               | 0                | .....         | .....            | .....         |
|              |                                                                                       |                              | 6.5             | 0                | .....         | .....            | .....         |
|              |                                                                                       |                              | .....           | ( <sup>2</sup> ) | .....         | .....            | .....         |

<sup>1</sup> Not determined.

<sup>2</sup> Five milligrams in 50 grams of the wet root.

Inspection of Table IV shows that many species besides loco plants contain barium in amounts not differing greatly from those found in the two loco genera, *Aragallus* or *Oxytropis* and *Astragalus*. Thus alfalfa (Nos. 1, 2, and 3), *Lupinus pusillus* (Nos. 12 and 14), *Lupinus*

<sup>1</sup> Hillebrand, W. F. Analysis of Silicate and Carbonate Rocks. Bulletin 305, U. S. Geological Survey, 1907, p. 116.

*sericeus* (No. 13), buffalo grass (No. 16), barley (No. 17), and millet (No. 18) contain barium in larger or small proportions. *Lupinus parviflorus* (No. 15), the grasses *Andropogon* (No. 19) and *Calamovilfa* (No. 22), the beet (No. 20), the yucca (No. 21), and the *Artemisia* (No. 23) contain none. Crawford also found barium in different botanical families.<sup>1</sup> While it is true that the average barium content of the loco plants is greater than that of the other plants, in many cases this difference is not great. Assuming that the barium is equally toxic wherever found, we would expect such plants as alfalfa (No. 1) and *Lupinus pusillus* (No. 12) to be more toxic under range conditions than such loco weeds as *Astragalus thurberi* (No. 8) and *Astragalus mollissimus* (No. 9), both of which contain far less barium. For this there is, of course, no evidence. In the laboratory, under certain quite arbitrary methods of preparing and feeding the extracts, both the alfalfa (No. 1) and the *Astragalus mollissimus* (No. 9) proved toxic, as may be seen by reference to protocols Nos. 5, 6, and 11<sup>2</sup> discussed in detail further on in this bulletin. These show that under artificial laboratory conditions no parallelism between barium content and toxicity was found. Hence, no evidence was discovered to corroborate older observations claiming that barium-free loco plants are not toxic.<sup>3</sup> Indeed, the loco poorest in barium was quite as toxic in the laboratory as the richest, as shown in the protocols.

#### OCCURRENCE OF BARIUM IN PLANTS FROM VIRGINIA.

As barium seemed to be present so frequently in plants occupying the same areas in Colorado as the loco plants, it seemed important to determine whether plants growing in other sections of the United States where barium occurs as a mineral also contain it. Pittsylvania, Campbell, Bedford, Tazewell, Russell, and Fauquier Counties in Virginia contain deposits of baryta, some of which have been developed commercially.<sup>4</sup> Plants were therefore collected at Evington in Campbell County, at Toshes and Pittsville in Pittsylvania County, at Gardners in Russell County, and at Catlett in Fauquier County. The barium determinations from plants thus collected gave the results shown in Table V.

<sup>1</sup> Crawford, A. C. Op. cit., p. 54.

<sup>2</sup> The protocols are collected at the end of this paper, pp. 56 to 61.

<sup>3</sup> Crawford, A. C. Op. cit., pp. 66-71.

<sup>4</sup> Watson, T. L. Geology of the Virginia Barite Deposits. Transactions of the American Institute of Mining Engineers, vol. 38, p. 710.

TABLE V.—*Barium content of Virginia plants from regions where barium occurs as a mineral.*

| Analysis No. | Plant, collection number, and locality.                                  | Moisture in air-dried plant. | In dried plant.  |                  | Barium sulphate in ash. |
|--------------|--------------------------------------------------------------------------|------------------------------|------------------|------------------|-------------------------|
|              |                                                                          |                              | Ash.             | Barium sulphate. |                         |
| 25           | <i>Andropogon virginicus</i> L. (broom sedge; A. 59, Gardners, Va.)..... | <i>Per cent.</i>             | <i>Per cent.</i> | <i>Per cent.</i> | <i>Per cent.</i>        |
| 26           | Grass (mainly crab-grass; A. 35, Pittsville, Va.).....                   | 6.01                         | 3.30             | 0.029            | 0.876                   |
| 27           | Soy bean (A. 36, Pittsville, Va.).....                                   | 6.16                         | 8.45             | .029             | .343                    |
| 28           | Hay (A. 19, Evington, Va.).....                                          | 8.10                         | 6.17             | .023             | .36                     |
| 29           | <i>Panicum sanguinale</i> L. (crab-grass; A. 49).....                    | 5.50                         | 5.53             | .014             | .26                     |
| 30           | <i>Andropogon virginicus</i> L. (broom sedge; F. 19, Evington, Va.)..... | 5.54                         | 6.60             | .010             | .147                    |
| 31           | Wheat (seed; A. 34, Pittsville, Va.).....                                | 5.72                         | 5.74             | .007             | .158                    |
| 32           | Maize (ear, leaf, and stem; A. 50, Toshes, Va.).....                     | (1)                          | ±2.00            | 0                | 0                       |
| 33           | Maize (ear, leaf, and stem; A. 61, Gardners, Va.).....                   | (1)                          | ±1.5             | 0                | 0                       |
|              |                                                                          | (1)                          | ±2.0             | 0                | 0                       |

1 Not determined.

Inspection of Table V shows that many of these plants contain barium. The soy bean plants from Pittsville and the hay from Evington contain as much barium as the loco plants from Colorado shown in Table IV. The soy beans grew in a field with cowpeas. The hay from this field was used year after year as fodder without the least ill effects. The field was several hundred yards from an old baryta working with a very small dump, but contamination from this source was impossible. The hay came from a field in the bottom just southwest of the railroad station at Evington, 2 miles from the nearest mine. It was taken from a barn where it had been stored for stock feed. Although the soils on which these plants grew contained barium, no condition resembling the loco-weed disease is known to have affected live stock in Virginia.

On the whole, a study of Tables IV and V does not show any very great selective absorption of barium by the loco plants. Obviously it is quite impossible to prove selective absorption by loco plants without a study of plants growing on the same plat. Variations in the soil, even within a limited area, might well be sufficient to account for the results. It was possible in several instances to examine plants growing adjacent to one another. Buffalo grass (No. 16), barley (No. 17), millet (No. 18), alfalfa (No. 3), and *Astragalus mollissimus* (No. 9) were all collected upon the same kind of soil less than one-quarter of a mile from one another. All of them contained barium. It is curious to note that the alfalfa (No. 3) and the *Astragalus* (No. 9) contain the least, while the buffalo grass (No. 16), one of the best forage grasses of the Plains, contains the most. The best (No. 20), the morning-glory root (No. 24), the yucca (No. 21), and *Astragalus mollissimus* (No. 7) were also collected very close to one another. Their barium content varies. *Artemisia* (No. 23) and the grass *Calamovilfa* (No. 22), neither of which contains barium, also grew

close together. The roots were removed from the buffalo grass (No. 16) before analysis because they contained soil. Of the beet (No. 20) and morning-glory (No. 24), only the roots were analyzed. In all other cases the entire plants were taken for analysis. The barley and the millet, although in seed, had not ripened.

It is possible that the barium found in these plants is not really present within them, but is derived from the earth and dust which cling to their exterior. As the writers were compelled to use plants gathered by others, who employed no special precautions in collecting them, it was necessary to take particular pains in the preparation of material for analysis. It is possible, too, that the differences in the barium content are due to the different degrees in which dust clings to different plants. While some of the plants studied were very hairy and would therefore retain much dust, others were quite smooth and would retain much less. To avoid these sources of error as far as possible, the plants were carefully cleaned and brushed before they were ground for analysis. It is not certain that this procedure removed all the earth, but such seemed to be the case. Moreover, only such plants were selected as seemed clean and free from soil. It was not practicable to wash the plants, for this leaches out salts and does not clean them well. The carefully cleaned plants were ground in a drug mill and thoroughly sampled. A considerable number of individual plants were always ground up together in order to obtain as fair a sample as possible. While these precautions may not have eliminated the sources of error, it does not seem likely that the latter entered materially into the results. The fact that in some instances the soil, as shown by the analyses of Prof. G. H. Failyer, of the Bureau of Soils, contained less barium than the ash of plants growing upon that soil is presumptive evidence that this metal was actually contained in the plant. Furthermore, the fact that the separate analyses of the leaves, the stems, and the roots of *Aragallus lamberti* (Nos. 34, 35, and 36) show the greatest amount of both ash and barium in the leaves (Table III) indicates the same fact, for if the presence of barium were due to contamination with soil the greatest amount ought to be in the root and the least in the leaf. The barium, however, accompanies the other salts, and it is well known that as a rule the actively growing leaves of plants contain greater amounts of ash than the roots and stems. *Aragallus* is no exception. These determinations of barium in the leaves also furnish, possibly, an explanation for the occasional variations in ash and barium content of the same species growing in the same locality. The results obtained for different specimens would be comparable only if the entire plants were always analyzed at the same stage of growth. The samples analyzed were not necessarily uniform in this respect. Against the evidence in

Table VI must be balanced the observation that the beet (No. 20), which was free from barium, was carefully peeled, and only the interior, which had never been in contact with the soil, was taken for analysis. The morning-glory root (No. 24), however, could not with absolute certainty be freed from soil.

TABLE VI.—*The distribution of barium in Aragallus lamberti (Pursh) Greene.*

| Analysis No. | Plant, collection number, and locality.                       | Moisture in air-dried plant. | In dried plant.  |                  | Barium sulphate in ash. |
|--------------|---------------------------------------------------------------|------------------------------|------------------|------------------|-------------------------|
|              |                                                               |                              | Ash.             | Barium sulphate. |                         |
|              |                                                               | <i>Per cent.</i>             | <i>Per cent.</i> | <i>Per cent.</i> | <i>Per cent.</i>        |
| 34           | <i>Aragallus lamberti</i> (leaves; M. 8.96, Hugo, Colo.)..... | 6.69                         | 22.02            | 0.034            | 0.156                   |
| 35           | <i>A. lamberti</i> (stems; M. 8.96, Hugo, Colo.).....         | 7.12                         | 9.10             | .027             | .298                    |
| 36           | <i>A. lamberti</i> (roots).....                               | 6.00                         | 7.92             | .020             | .258                    |

With the plants from Virginia further precautions were possible. In gathering them each plant or tuft of grass was carefully cut off well above the soil. The soy beans were further sorted in the laboratory and only the clean tops used. The pods had formed, but the seeds were not quite ripe. As the plants were erect and about 18 inches tall, the chance that soil contaminated the tops is small. The hay from Evington was taken from a barn.

At any rate, whatever the source of the barium, it reaches the intestines of the stock browsing upon such forage plants as contain it, so that the possibility of its affecting them must be considered.

#### SOLUBILITY OF BARIUM IN LOCO PLANTS.

Some of the salts of barium, particularly the sulphate and silicate, are very insoluble. The sulphate is so insoluble that it is believed to be quite harmless, though the soluble salts of barium are quite toxic. Indeed, baryta, because it is supposed to be harmless and is very white and very heavy, is said to be used as an adulterant in many articles of commerce, sometimes even in articles of diet. Hence, it is important to know whether the barium present in these plants is soluble. We have not found it appreciably soluble in water in any dried loco plants examined, which corroborates some of the observations of Crawford.<sup>1</sup> Following his procedure, extracts were prepared by means of peptic and tryptic digestion.<sup>2</sup> The finely ground plant was percolated with about 10 liters of water, saturated with chloroform to prevent putrefaction. The residue was then digested at 38° C. for 12 to 24 hours with a solution of pepsin in 0.15 per cent hydrochloric acid to which much toluol and chloroform

<sup>1</sup> Crawford, A. C. Op. cit., p. 68.

<sup>2</sup> Crawford, A. C. Op. cit., pp. 66-71.

had been added. The liquid was filtered off and added to the percolate; the residue was digested a second time with a solution of trypsin in 0.2 per cent sodium carbonate containing much chloroform and toluol. The liquid was filtered from the residue and also added to the percolate. The combined extracts were then neutralized and concentrated under diminished pressure, a difficult and painstaking task because of the foaming. The residues were dried and preserved for analysis. Barium was determined separately in the dry residues and the concentrated extracts. The results are given in Table VII.

TABLE VII.—*Determination of barium in the extract obtained by digestion of loco weeds and other plants and in the residue from such digestions.*

| Analysis No. | Plant, collection number, and locality.                               | Barium sulphate in 100 grams— |                                                                | Barium sulphate in the extract of 100 grams dry plant. |
|--------------|-----------------------------------------------------------------------|-------------------------------|----------------------------------------------------------------|--------------------------------------------------------|
|              |                                                                       | Of the dry plant.             | Of the dried residue after digesting and extracting the plant. |                                                        |
| 37           | Alfalfa (M. 9.65, Limon, Colo.) .....                                 | 0.010                         | (1)                                                            | 0.0074                                                 |
| 38           | <i>Astragalus thurberi</i> Gray (Tm. 3, Calabazos, Ariz.) .....       | .009                          | 0.010                                                          | .0001                                                  |
| 39           | <i>Astragalus mollissimus</i> Torr. (M. 9.195, Hugo, Colo.) .....     | .016                          | (1)                                                            | .0006                                                  |
| 40           | <i>Aragallus lamberti</i> (Pursh) Greene (M. 8.96, Hugo, Colo.) ... { | .023                          | .043                                                           | .0016                                                  |
|              |                                                                       | .028                          | .051                                                           |                                                        |
| 41           | <i>Astragalus nitidus</i> Dougl. (Woodland Park, Colo.) .....         | .043                          | .052                                                           | .004                                                   |
|              |                                                                       | .042                          |                                                                |                                                        |
| 42           | Alfalfa (M. 90, Hugo, Colo.) .....                                    | .014                          | .014                                                           | (1)                                                    |
| 43           | Soy bean (A, Pittsville, Va.) .....                                   | .023                          | .033                                                           | (1)                                                    |
| 44           | <i>Astragalus simplicifolius</i> Gray (M. 9.4, Buckman, Wyo.) .....   | .022                          | .029                                                           | (1)                                                    |
|              |                                                                       | .029                          |                                                                |                                                        |
| 45           | <i>Astragalus mollissimus</i> Torr. (M. 9.95, Hugo, Colo.) .....      | .032                          | .029                                                           | (1)                                                    |
|              |                                                                       | .029                          |                                                                |                                                        |

<sup>1</sup> Not determined.

Inspection of Table VII shows that the barium content of the insoluble part of the plants was either relatively unchanged or relatively markedly increased. The increase is due to the fact that the extraction had removed much soluble material, concentrating the barium in the insoluble residue. There is one record in the literature of a barium determination in a residue left after an extraction by digestion of this type,<sup>1</sup> and in it no barium was found. When one considers that at times in the exact determination of such small quantities of barium the precipitate may be unusually slow in forming, it is evident that no great weight need necessarily be placed upon a single experiment. The writers, particularly in the beginning of their work, had an occasional misleading determination, as duplicate analyses showed. As is evident from an inspection of Table VII, these results were controlled in five instances by analyzing the extracts obtained by digestion. The extracts were evaporated to a sirup,

<sup>1</sup> Crawford, A. C. Op. cit., p. 67.

transferred to platinum, and ashed. Barium was determined in the ash in the manner recommended by Hillebrand.<sup>1</sup> The amount of barium was very small, hardly more than a trace. In the extract from 100 grams of the dry plant it varied from a maximum of 0.0074 gram barium sulphate in the alfalfa to a minimum of 0.0001 gram barium sulphate in the *Astragalus thurberi*. The source of these traces of barium is not quite clear, for the only three extracts tested for soluble sulphates, *Astragalus thurberi* (No. 38), *Astragalus mollissimus* (No. 45), and alfalfa (No. 37), contained appreciable amounts, certainly more than enough to convert all the barium into sulphate. Moreover, it was not easy to filter the extracts absolutely clear, and it was difficult, owing to their dark color, to know when they were clear. The extract of *Aragallus lamberti* (No. 40) was filtered through ordinary folded filters. It is not certain, therefore, that traces of precipitated barium sulphate did not pass through the filter. *Astragalus thurberi* (No. 38), *Astragalus mollissimus* (No. 39), and alfalfa (No. 37) were filtered through Schleicher and Schüll quantitative filters No. 590. While it is possible, although unlikely, that the traces of barium found in the extracts may have been due to imperfect filtration, there is another and more probable explanation dependent upon the normal solubility of barium sulphate. This salt is soluble in distilled water to the extent of about 2.5 milligrams to the liter.<sup>2</sup> As a number of liters were used for each extraction, the surprising thing is not that traces of barium were found, but that there was so little. Probably the explanation is that either the small amount of barium present in the air-dried plant occurs in combination with silica which is abundantly present or that other substances in the solution affect the solubility. Perhaps there are traces of organic compounds of barium. In this connection it is interesting to note that the alfalfa, which was relatively poor in ash and silica and rich in calcium, yielded more barium to the extract relatively and absolutely than the loco plants. These determinations of the barium content of extracts of air-dried plants are, as far as known to the writers, the only ones recorded in the literature of the subject.<sup>3</sup> Indeed, they are not aware that anyone before them has ever tested toxic loco extracts for barium.<sup>3</sup> It has, to be sure, been suggested that plants might be found in which the barium is not extracted by digestion.<sup>4</sup> The writers have found such plants and no others. Fresh plants they have not examined.

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<sup>1</sup> Hillebrand, W. F. Loc. cit.

<sup>2</sup> Fresenius, C. R. Quantitative Chemical Analysis, vol. 1, New York, 1908, p. 169.

<sup>3</sup> Crawford, A. C. Op. cit., pp. 67-68.

<sup>4</sup> Crawford, A. C. Op. cit., p. 70.

## RELATION OF BARIUM TO TOXICITY.

All these observations argue against the specific toxic action of barium in loco plants. The amounts of barium present in the extracts were too small to prove fatal; in fact, they were hardly more than traces. It has, moreover, been shown that this metal is present in some insoluble form, probably as sulphate or silicate. From the extract of 200 grams of *Aragallus lamberti* (No. 40) only 0.0032 gram barium sulphate, corresponding to 0.0023 gram barium chlorid, was obtained. Now Crawford has shown that the extract obtained from 200 grams of air-dried *Astragalus mollissimus* by digestion is the toxic dose for a rabbit of about 1 kilogram,<sup>1</sup> an observation which the writers can completely corroborate. The toxic dose of barium chlorid when given in one or two feedings lies between 0.1 and 0.2 gram.<sup>2</sup> The toxic dose of extracts fed in this investigation contained from one-fiftieth to less than one-hundredth as much barium as this. The death of rabbits in 5 to 16 days from the feeding of smaller amounts of barium chlorid (0.025 gram daily) has been reported.<sup>2</sup> The extracts prepared by the writers contained from one-fiftieth to less than one-tenth of these small, slowly killing doses. Nevertheless, these extracts were very toxic, proving fatal within 24 hours. Rabbits were killed by feeding extracts of from 200 to 250 grams of air-dried *Astragalus arizonicus*, *Astragalus thurberi*, *Aragallus lamberti*, *Astragalus mollissimus*, and *Astragalus nitidus* in a single dose or in two divided doses separated by an interval of 24 hours, as may be seen by reference to protocols Nos. 1, 2, 7, 11, and 19. Furthermore, similar extracts of alfalfa were often fatal in a like way. Of four experiments with alfalfa, as shown in protocols Nos. 3, 5, 6, and 8, fatal results were recorded three times.

It will be seen from Table VII that the soy bean from Virginia behaved in a similar way as regards solubility of its barium. It did not yield more than traces of barium on extraction. It differed, however, in not yielding a toxic extract, as shown by protocol No. 20. The dosage fed was somewhat less than in the experiments with the loco plant. Moreover, as but one animal experiment was performed, the lack of toxicity of Virginia plants under these artificial laboratory conditions is not proved absolutely. Barium in dried Virginia and Colorado plants is present in an insoluble form. If, nevertheless, it is a cause of loco poisoning the same trouble is to be expected with Virginia cattle. No evidence of such trouble could be discovered. This is the more significant as Russell and Tazewell Counties, Va., are very successful grazing and cattle-growing districts. Of course this is not conclusive. It is still possible that the barium is

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<sup>1</sup> Crawford, A. C. Op. cit., p. 46.

<sup>2</sup> Crawford, A. C. Op. cit., pp. 57 ff.

present in different combinations in the different plants. Such a difference, if present, could hardly be the result of a difference in the barium compounds of the soil, for both in Virginia and Colorado the barium occurs in a form in which it is but very slightly soluble in dilute acid.<sup>1</sup> Moreover, Marsh reports that the waters in the region from which the tested plants came contain much sulphate, which would certainly convert the barium into the sulphate.

The possibility that the barium is harmless in all the plants in which it occurs except the loco plants, because it is physiologically antagonized by other salts or substances in all of them except in the loco plants, is conceivable though not probable. To test this possibility would involve a long research, which does not seem worth the labor in view of the fact that the toxic extracts obtained in the laboratory contain but traces of barium and in view of the equally significant fact that some alfalfa extracts are under the same conditions quite as toxic.

#### TOXICITY OF ASH EXTRACTS.

As the extracts contained only traces of barium and as so harmless a plant as alfalfa was, under the extreme conditions of the laboratory experiments, often quite as toxic as loco plants, it seemed necessary to analyze the toxic action further. This was rendered easier by Crawford's discovery that the acetic-acid extracts of the ash of loco plants, freed from the excess of acid, were fatal when fed to rabbits.<sup>2</sup> This experiment was repeated with the specimen of alfalfa which contained almost no barium. As will be seen by reference to protocol No. 8, the extract prepared from this plant by digestion was very toxic. In a sample of the ash of 600 grams of this plant, barium sulphate and calcium oxid were determined, showing 0.003 per cent barium sulphate and 1.47 per cent calcium oxid in the dry plant. The remainder of the ash was extracted with an excess of acetic acid. The extract was made up to a known volume and a portion equivalent to 200 grams of dry alfalfa taken. This was dried down on the water bath to remove the acetic acid, and the residue was baked for half an hour at 150° C. in the oven to remove any acid remaining. After cooling, the residue was suspended in water. It was not completely soluble, probably because of the coagulation of silicates; perhaps, also, because of the formation of basic salts. The reaction was slightly alkaline. This milky-white liquid, containing some insoluble matter in suspension, was fed to a rabbit in two doses, one in the forenoon and one in the afternoon, with fatal results, as shown in protocol No. 8. The liquid, a portion of which was fed in this experiment, contained no barium. The amount

<sup>1</sup> Privately communicated by Prof. Failyer.

<sup>2</sup> Crawford, A. C. Op. cit., p. 47 ff.

fed contained 11.2 grams calcium acetate. Consequently, in the experiment 11 grams of calcium were fed in addition to any other soluble salts that may have been present in the ash. Now, 5 grams of calcium acetate have been reported sufficient to kill a rabbit weighing 652 grams.<sup>1</sup> This the writers have been able to confirm. Rabbits have been killed with corresponding doses of calcium chlorid as recorded in protocols Nos. 16, 17, and 18. Calcium chlorid was used instead of the acetate because, being an inorganic compound, it can be easily dried, so that the dosage is easier than with the acetate. The few experiments performed render it probable that the fatal dose for rabbits lies between 4 and 5 grams calcium chlorid per kilogram of body weight when this amount is administered dissolved in about 60 to 100 cubic centimeters of water in two equal doses separated by an interval of three or four hours. Five grams calcium chlorid contain as much calcium as do 7.118 grams calcium acetate.

Evidently there is in these alfalfas, under the laboratory conditions in which the ash of loco plants has been reported toxic,<sup>2</sup> enough calcium to kill animals without considering the toxic action of other salts contained in the plants, such as potassium. However, experiments with the acetic-acid extracts of ashed loco plants did not yield the same results. Though the extracts were made in the same way as that of the experiment with alfalfa given in protocol No. 8, they did not prove fatal, as appears in protocols Nos. 9, 10, and 13. Like the extract of the alfalfa ash, they contained no barium. No record that such extracts have ever been tested for barium could be found. Their ash seemed to contain less calcium and less soluble matter generally than the alfalfa ash. In protocol No. 10 the total amount of calcium acetate obtained in the extract of the ash of 300 grams of air-dried plant was 8.5 grams, equivalent to 3.9+ grams per kilogram of body weight. The calcium acetate of the experiment with alfalfa ash amounted to a dosage of 4.9 grams per kilogram of body weight. The calcium fed in the loco-ash experiment No. 10 was well below the lethal dose, while that found in the alfalfa-ash experiment approached the lethal limits. The loco plants ashed were such as yielded toxic extracts by the digestion method. That in these experiments, unlike those previously reported,<sup>3</sup> the ash of these loco plants failed to yield fatal extracts may be due partly to the fact that larger rabbits were used and partly to the fact that the extract was fed in two doses separated by an interval of a few hours. In the fatal cases reported<sup>3</sup> the whole extract seems usually to have been fed in a single dose. This was heroic treatment to which

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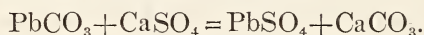
<sup>1</sup> Crawford, A. C. Op. cit., p. 52.

<sup>2</sup> Crawford, A. C. Op. cit., p. 47 ff.

<sup>3</sup> Crawford, A. C. Op. cit., p. 48 ff.

it did not seem wise to resort, particularly as the loco plants are supposed to have cumulative action. The experience of the writers has been that an amount of calcium which may be fatal when administered in a single dose need not necessarily prove so when administered in divided doses at short intervals. This is quite in accord with the reports of experiments with the ash extracts.<sup>1</sup> In one case,<sup>2</sup> in which the extract was fed in two doses on two successive days, the rabbit died on the fourteenth day. In another case, in which a large rabbit was used, the extract from the ash of 200 grams of the plant did not prove fatal.<sup>2</sup> The facts that the writers failed to get fatal results with the ash extract of loco plants, that they did get fatal results with alfalfa-ash extract containing more salts than the loco-ash extracts, that none of the ash extracts contained barium, and that there is no record in the literature that they ever do contain barium furnish presumptive evidence that such fatal results as may be obtained in experiments of this kind are due rather to calcium and perhaps to other salts than to barium.

If this line of reasoning is correct, then the reason why in the past nontoxic extracts were obtained from the ash when the extract was made with dilute sulphuric acid instead of acetic acid becomes clear.<sup>3</sup> By the former procedure the calcium was converted into the insoluble sulphate, in which form only a small fraction of it would pass into the extract. When the extract was then treated with lead carbonate to remove the excess of sulphuric acid, any calcium sulphate present in the solution would react thus:



As calcium carbonate is very insoluble, practically all the calcium would be removed from the solution. These experiments indicate that the removal of the calcium must have made the solution harmless, for in this investigation barium was never found in the acetic-acid extracts of the ash.

It has been stated<sup>4</sup> that the extract of loco-weed ash made with 94 and 70 per cent alcohol proved toxic to rabbits in two cases. While this experiment was not repeated in this investigation, it is worth while in this connection to call attention to the fact that many potassium salts and also calcium oxid are fairly soluble in watery alcohol.

#### TOXICITY OF ALFALFA EXTRACTS.

The toxicity of the extracts of dry alfalfa is probably not dependent to so great an extent upon the calcium as is the case with

<sup>1</sup> Crawford, A. C. Op. cit., p. 48 ff.

<sup>2</sup> Crawford, A. C. Op. cit., p. 49.

<sup>3</sup> Crawford, A. C. Op. cit., pp. 52-53.

<sup>4</sup> Crawford, A. C. Op. cit., pp. 48-49.

the ash extracts, for the extract from the dry alfalfa plant does not contain as much calcium as the acetic-acid extract made from the ash. This was the case with the alfalfa used in protocol No. 8, described on page 58. As there shown, the acetic-acid extract of the ash contained 11.2 grams of calcium acetate. The same plant yielded but 3 grams of calcium calculated as acetate in the extract prepared by digestion.

The calcium in the extract was determined by incinerating the extract, digesting the ash with hydrochloric acid, and filtering. To the filtrate sulphuric acid and alcohol were added, and the calcium-sulphate precipitate was washed with dilute alcohol. The washed calcium sulphate was fused with sodium carbonate and the melt was dissolved in water and filtered. The residue of calcium carbonate was dissolved in dilute hydrochloric acid and precipitated with ammonium oxalate and ammonia. The precipitate was filtered off, washed, and ignited to constant weight over the blast lamp.

The alfalfa extract, which contained but 3 grams of calcium calculated as acetate, did not prove poisonous to a rabbit weighing 1,950 grams, as evidenced by protocol No. 3. Evidently the calcium in alfalfa behaves in a way similar to that of the barium in the loco plants; only a portion is extracted by digestion, at least in the one case examined. The alfalfa extracts which proved toxic in protocols Nos. 5 and 6 were given in somewhat larger dosage. Unfortunately their calcium content was not determined. It would seem that in these cases there was probably a summation of toxic actions; the action of calcium, potassium, and other salts, and perhaps the action of constituents as yet unstudied. The improbability of a physiological antagonism between salts playing a rôle has been pointed out. The alfalfa extracts differed from the loco-weed extracts in containing very much greater amounts of organic acids. When fed to rabbits they produced prompt diuresis and sometimes the passage of a moderate quantity of feces, effects not often noticed with loco-weed extracts. Perhaps these effects were due to the organic acids present.

It is interesting to note in this connection that the nontoxic extract of the Virginia soy bean already referred to differed markedly from the extracts of western plants in the very much smaller amounts of material extracted.

#### TOXICITY OF LOCO-WEED EXTRACTS.

The toxicity of the loco-weed extracts is perhaps dependent upon different causes, for the calcium in the one case studied seems to be more readily extracted than in the one sample of alfalfa studied. A weight of 100 grams of *Astragalus mollissimus* (analysis No. 9) yielded an extract containing 0.8514 gram calcium oxid, while the

ash from the same quantity of plant yielded when extracted with acetic acid only 0.7864 gram calcium oxid. It is not justifiable to generalize from these two experiments, but so far as the evidence goes they seem to show that whereas in alfalfa only a portion of the calcium may be removed by extraction, in the loco weeds relatively more may be removed in this way. After ashing, the reverse seems to be the case.

If it is justifiable to conclude that calcium and probably other salts play an important part in the toxicity of these extracts prepared by digestion, this may be a clue to the reason why more toxic extracts are obtained by digesting with artificial gastric and pancreatic juices.<sup>1</sup> The hydrochloric acid of the gastric juice would render soluble the calcium present in the form of phosphate, carbonate, oxalate, and perhaps other insoluble compounds. If any of the calcium be present in the form of the insoluble salts of organic acids, the latter might also pass into solution. Now some organic acids, such as oxalic, are themselves quite toxic and, if extracted, would add their action to that of the other toxic constituents of the extract. To test the influence of the method of digestion upon the calcium content of the extract the writers determined separately (1) the calcium in the water extract, (2) the calcium in the extract obtained by digesting with artificial gastric juice the residue from the water extraction, and, finally, (3) the calcium in the extract obtained by the tryptic digestion of the residue from the gastric digestion. The results were as follows:

| <i>Calcium oxid.</i>                                                                               |  | Gram.  |
|----------------------------------------------------------------------------------------------------|--|--------|
| (1) Water extract of 100 grams <i>Astragalus mollissimus</i><br>(M. 9.45)-----                     |  | 0.7105 |
| (2) Peptic extract of the residue from (1) (19.5 per cent<br>of total calcium oxid extracted)----- |  | .1383  |
| (3) Tryptic extract of the residue from (2)-----                                                   |  | .0026  |

As will be seen, more than one-sixth of all the calcium extracted was removed by peptic digestion. This is a quantity large enough to account for the greater toxicity of the extracts obtained by digestion.

#### COLLOIDAL BARIUM SULPHATE.

While the fact that by no method of digestion with an aqueous menstruum could extracts containing more than traces of barium be obtained renders it exceedingly improbable that the toxic action depended in any way upon barium, still it is conceivable that the traces of barium sometimes found in the extracts may have been present in some colloidal form far more active than any known compound of

<sup>1</sup> Crawford, A. C. Op. cit., pp. 66-67.

barium. Improbable though this assumption be, it was tested by preparing colloidal solutions of barium sulphate according to the method given by Feilmann.<sup>1</sup> A rabbit was fed 30 cubic centimeters of colloidal barium-sulphate solution the first day and 50 cubic centimeters on each of the two succeeding days, 6.5 grams colloidal barium sulphate in all, without any visible effects (protocol No. 21). While a single experiment of this sort is never finally conclusive, in view of the negative results obtained it did not seem worth while carrying this line of investigation further. The possibility of minute traces of barium acting in some occult way becomes still more improbable if one considers that the toxic effects of the ash extracts are amply accounted for by the other salts present. Moreover, the ash extracts did not contain even traces of barium. In the extracts of the unashed plants the cause of the toxic action is not so clear. These extracts were very concentrated and we may have had to deal with the action of other substances besides the salts. It is possible that they contain, in addition to the salts, some other toxic factor or factors. Crawford also considers it possible that the toxic action is the resultant of the action of the total constituents of the plant.<sup>2</sup>

All this work and the greater part of the work of Crawford was done with dry plants. While it has been shown that the dried plants used in this investigation do not contain appreciable amounts of barium in a soluble form, it is quite possible that it is otherwise with fresh plants. On this point the writers have no evidence. It is, however, exceedingly improbable, for Marsh<sup>3</sup> was able to produce the loco-weed disease in cattle with the dried plant in the same dosage, making due allowance for water lost in drying, and in the same length of time as with the fresh plant.

## RESULTS OF PLANT ANALYSES AND TOXICITY EXPERIMENTS.

All this evidence, viz, the insolubility of the barium in dried loco plants, its presence in other species of Colorado plants, its presence in plants from Virginia, where the loco-weed disease is unknown, and the presence of large quantities of other salts in the extract, merely shows the inadequacy of the laboratory evidence connecting barium with the toxic action of the dry plants. These arguments can not be applied to the conditions of the range without further experimentation. Certainly the calcium and other salts which are so important a factor in the inspissated extracts prepared in the laboratory have nothing to do with the toxicity of these plants under range conditions. Moreover, while peptic and tryptic digestion in

<sup>1</sup> Feilmann, E. Colloidal Barium Sulphate. Chemical News, vol. 98, 1908, p. 310.

<sup>2</sup> Crawford, A. C. Op. cit., p. 70.

<sup>3</sup> Marsh, C. D. Privately communicated.

vitro does not lead to extracts containing more than traces of barium, conditions in the alimentary tract of cattle may be quite different. There unknown factors may conceivably come into play which perhaps cause barium to be absorbed. However much one may be inclined to doubt this possibility, without adequate experimental evidence, it is not right really to deny it. Before attacking this question it would be important to investigate whether the barium, which, as has been shown, is probably present in the plant in an insoluble form, is absorbed and enters into the metabolism of cattle.

#### ANALYSES OF THE BONES OF LOCOED ANIMALS.

Two ways of testing the problem whether the barium is absorbed presented themselves. One was to search for barium in the urine of animals upon a loco-weed diet. This was not a very promising line of attack, because the greater part of any barium that may be absorbed is excreted with the great bulk of the calcium in the feces, where it could not be distinguished from the unabsorbed barium.<sup>1</sup> The other line of attack was to analyze the carcasses of animals dead from the loco-weed disease. This the writers have not been in a position to do as completely as would be desirable. Mendel and Thacher<sup>2</sup> have, however, shown that the bones are storehouses of the earthy metals, especially strontium. Analogous observations have been made by König<sup>3</sup> and Lehnerdt.<sup>4</sup> The bones from two of the animals experimented upon by Marsh were accordingly secured and analyzed for barium, with absolutely negative results. Unfortunately, the animals had been dead for a year and their bones had been bleaching for that length of time upon the prairie. It may be that the barium had been removed by exposure to the weather, but in the presence of so much calcium this is not likely.

No. 10 was a steer, weighing 820 to 900 pounds, which had eaten some loco but had never been distinctly locoed. It had then received 1,437 grams of barium chlorid, 1/313 of its body weight, in divided doses from May 30 to September 10, when it succumbed.<sup>5</sup> Animal No. 55 was a horse that died of loco poisoning.<sup>5</sup> Barium was searched

<sup>1</sup> Meyer, G. M. The Elimination of Barium. *American Journal of Physiology*, vol. 25, 1909, p. 142.

<sup>2</sup> Mendel, L. B., and Thacher, H. C. The Excretion of Strontium. *American Journal of Physiology*, vol. 11, 1904, p. 15.

<sup>3</sup> König, J. Substitution des Kalkes in den Knochen. *Zeitschrift für Biologie*, vol. 10, 1874, p. 69.

<sup>4</sup> Lehnerdt. Ueber den Einfluss des Strontium auf die intrauterine Entwicklung des Knochensystems. *Münchener Medizinische Wochenschrift*, vol. 56, 1909, p. 679. (Bericht des Vereins der Aerzte in Halle, a. S.) Lehnerdt. Strontiumfütterung an säugende Tiere. *Münchener Medizinische Wochenschrift*, vol. 56, 1909, p. 1976. (Bericht des Vereins der Aerzte in Halle, a. S.)

<sup>5</sup> The full history of this animal may be found in "The Loco-Weed Disease of the Plains," by C. D. Marsh. *Bulletin 112, Bureau of Animal Industry, U. S. Dept. of Agriculture*, 1909, and Part I of this bulletin, pp. 19-21.

<sup>6</sup> Marsh, C. D. Part I of this bulletin, p. 14.

for as follows: The ash from 10 grams of the bones was fused with sodium carbonate, the melt dissolved in water, the insoluble residue filtered off and dissolved in weak hydrochloric acid, and the clear solution treated with a saturated aqueous solution of calcium sulphate. Not even a trace of barium could be detected. It is possible, of course, that this method was not suited to the end in view, but in control experiments in which traces of barium salts were added the latter were readily detected by this method.

#### DISCUSSION OF BONE ANALYSES.

If it be assumed that the failure to find barium in the bones of the locoed animal as well as in the bones of the animal fed soluble barium salts for months is reliable, it does not argue that barium can produce a chronic intoxication. Were the action of barium cumulative, it is likely that some trace of barium would remain in the bones. This is in accord with Marsh's<sup>1</sup> experiments, in which he was unable to produce chronic intoxication by feeding barium over long periods. As far as our incomplete evidence goes, it does not argue for a long-continued storage of barium in the organism.<sup>2</sup> However, even if barium had been found in the bones, this fact would not prove that there was any connection between its occurrence there and loco poisoning. Thus, if the methods used be sufficiently delicate, arsenic may be found in many living things which never show symptoms of arsenical poisoning.

#### SUMMARY AND CONCLUSIONS.

The etiological factor in the loco weeds which is responsible for the toxicity of these plants in laboratory experiments is not barium. This conclusion is based upon the following evidence:

Many kinds of plants from the Western States contain barium in amounts that are of the same order of magnitude as in loco plants. There is no evidence that such plants injure stock upon the range.

Many plants in certain sections of Virginia contain barium in quantities that are of the same order of magnitude as in loco plants. There is no evidence that such plants injure stock.

In dried loco plants barium is contained in an almost insoluble form. Even extracts prepared by digesting successively with artificial gastric and pancreatic juice contain but slight traces. Nevertheless, in large doses these extracts may be toxic.

There are usually enough salts of calcium, potassium, and metals other than barium in the extract of 250 grams of dried loco plant, the usual toxic dose for rabbits, to account for death.

<sup>1</sup> Marsh, C. D. Part I of this bulletin, p. 37.

<sup>2</sup> Mendel, L. B., and Sicher, D. F. The Excretion of Barium. *American Journal of Physiology*, vol. 16, 1906, p. 151.

Similar extracts of alfalfa may also cause death of rabbits in similar dosages.

The acetic-acid extracts of the ash of such barium-yielding plants from the Western States as were examined contain at most but minute traces of barium.

### PROTOCOLS.

#### EXPERIMENT No. 1.

*Astragalus arizonicus* Gray; type specimen (Tm. 721) deposited in the economic herbarium; collected March 30, 1908, on hills east of Nogales, Ariz. Of the air-dry plant, 500 grams were ground in a drug mill and thoroughly percolated with chloroform water till the extract was a light straw color. The undissolved residue was digested for six hours at 38° to 40° C. with artificial gastric juice in the presence of chloroform toluol. The liquid was filtered off and after being neutralized was combined with the percolate. The undissolved residue was digested six hours with trypsin in 0.2 per cent sodium carbonate, in the presence of chloroform and toluol. This liquid was also neutralized and combined with the percolate. The combined extracts were then concentrated in vacuo to 375 cubic centimeters and carefully neutralized with sodium carbonate. The concentrated extract contained a precipitate in suspension.

January 19, 1909. After stirring so as to suspend the precipitate in it evenly, 40 cubic centimeters of the liquid were fed, at 4 p. m., to a rabbit weighing 1,475 grams.

January 20. The rabbit had severe diarrhea and passed much urine during the night. At 12.20 p. m. it was fed 50 c. c. of the liquid. At 12.45 it was dead. The total amount fed corresponded to 130 grams of plant. The autopsy at 3.45 p. m., showed the stomach distended; its vessels were injected, mainly at the lesser curvature. The greater part of the mucous membrane was only moderately congested; the congestion was considerable only at the fundus. No ulcers. Just beyond the pylorus the small intestines were moderately congested; otherwise normal, except about the middle of the ileum, where some congestion and several hemorrhages were found. One hemorrhagic spot on colon.

#### EXPERIMENT No. 2.

*Astragalus thurberi* Gray: type specimen (Tm. 807) deposited in economic herbarium; collected at Patagonia, Ariz., April 6, 1908. Of the air-dry plant, 500 grams were ground, percolated, and extracted as in experiment No. 1. The extracts were concentrated to a volume of 225 cubic centimeters and made slightly alkaline with sodium carbonate.

January 27, 1909. A male rabbit weighing 1,700 grams was fed 40 cubic centimeters of the extract without apparent effect.

January 28. The rabbit weighed 1,590 grams. Again fed 40 cubic centimeters. No marked symptoms.

January 29. The rabbit weighed 1,449 grams. Fed 50 cubic centimeters. Effect about as on previous day. No serious symptoms.

February 2. Rabbit weighed 1,560 grams. The animal was fed 50 cubic centimeters; soon moribund, apparently paralyzed, but reacted to stimuli. It died in 50 minutes, having a terminal convulsion. Autopsy three hours after death showed intestines much distended with gas. There seemed to be an abundant secretion of fluid in them. The stomach contained much mucus and

a few semisolid fecal pellets. Mucous membrane much congested at the great and less so at the small curvature. Pylorus free from congestion. Some small hemorrhages in the congested areas. No ulcers.

EXPERIMENT No. 3.

Of alfalfa collected 30 miles south of Hugo, Colo., 500 grams were ground and extracted as in the previous experiments. The evaporation of the extract in vacuo had to be abandoned because of foaming. It was therefore evaporated on the water bath to a thin sirup. The extract was then neutralized with sodium carbonate. It was so acid that some of it was lost because of the foaming. It was warmed on the water bath to drive off dissolved carbonic acid. The solution remaining had a volume of 200 cubic centimeters.

February 10, 1909. Fifty cubic centimeters were fed to a large rabbit. The animal soon seemed uncomfortable, breathed rapidly, lay stretched out, and urinated profusely. In an hour these symptoms had passed off and the animal seemed normal, though quiet.

February 11. The feeding was repeated with the same effect.

EXPERIMENT No. 4.

*Astragalus diphyus* Gray; type specimen (Tm. 907) deposited in the economic herbarium; collected 10 miles east of Jerome Junction, Ariz. Of the air-dry plant 500 grams were extracted as in experiments Nos. 1 and 2. The combined extract was concentrated in vacuo to 250 cubic centimeters.

February 23, 1909. Of the concentrated neutralized extract 45 cubic centimeters were fed in the afternoon to a large rabbit.

February 24. A like amount fed in the forenoon. The rabbit seemed somewhat prostrated. A third similar dose fed in the afternoon. Death in 90 minutes. No autopsy.

EXPERIMENT No. 5.

An extract of 500 grams of alfalfa, collected 30 miles south of Hugo, Colo., was prepared as in experiment No. 3. About half of the resulting sirup was fed August 30, 1909, to a rabbit weighing 1,885 grams. The animal urinated much and passed some feces. It died in  $3\frac{1}{2}$  hours. No autopsy.

EXPERIMENT No. 6.

September 1, 1909. A rabbit weighing 1,880 grams was fed 50 cubic centimeters of the extract used in experiment No. 5. It urinated, passed some feces, and was dead in  $2\frac{3}{4}$  hours. On autopsy the intestines were found almost empty. The stomach was much injected; at the fundus its mucous membrane was congested and inflamed and showed two small ulcers; the pyloric end was free and pale. Heart not dilated; full of clot on both sides.

EXPERIMENT No. 7.

An extract was made from 500 grams of *Aragallus lamberti* (Pursh) Greene (collection No. 8.96) from Hugo, Colo. The extract was neutralized and concentrated in vacuo to 150 cubic centimeters. On July 25, 1909, 90 cubic centimeters were fed to a rabbit weighing 1,485 grams. Death in two hours. The remaining 60 cubic centimeters were analyzed and yielded only 0.0031 gram

barium sulphate. The residue of the plant was also analyzed and shown to contain a larger percentage of barium than before extraction. (Cf. Table VII.)

#### EXPERIMENT No. 8.

A quantity of 400 grams of alfalfa (collection No. M. 90) was ashed in platinum. The ash was extracted with dilute acetic acid and the extract evaporated to dryness in porcelain. The residue was a white, crummy mass. It was heated to 150° C. in the oven to remove any remaining traces of acetic acid. It was then treated with 150 cubic centimeters of water. A small part remained undissolved, so that the liquid was milky in appearance.

September 2, 1909. Fifty cubic centimeters of this milky suspension were fed at 11.15 a. m. to a rabbit weighing 2,250 grams. The animal breathed rapidly, lying on its side with hind legs extended. It soon urinated, sitting up for the purpose and then lying down again. The effects gradually passed off, so that by 1 p. m. it seemed normal. Did not eat. At 2.45, 60 c. c. were fed. For a time this did not seem to have much effect, but at 4.30 p. m. the rabbit was lying on its side prostrated. It passed some feces, but not very much. At 5.10 p. m. it was hardly able to raise its head or move its hind legs. Breathing rapid and shallow. At 5.15 respiration ceased. The heartbeat could still be seen and felt for a time after respiration had ceased. The amount of extract fed corresponded to the ash of 210 grams of plant. It was distinctly alkaline.

Autopsy, September 3. The abdominal cavity contained about 50 c. c. of bloody serous fluid. Animal pregnant. Bladder empty and contracted. Spleen normal. Stomach very large, filled with puttylike food mass, except at pyloric end; mucous membrane of greater curvature, a livid purple, and greatly congested; a few slight erosions; very little autolysis; two distinct hemorrhagic areas, each the size of a small pea.

#### EXPERIMENT No. 9.

An acetic-acid extract was made of the ash of 300 grams of *Aragallus lamberti* (Pursh) Greene, collected at Hugo, Colo., in the manner described in protocol of experiment No. 8. The volume of the extract was about 100 c. c.

November 28, 1909. Half of this extract was fed at 12.20 p. m. to a rabbit weighing 2,050 grams without producing much effect. The remainder was fed at 5.15 p. m. without much effect. The next morning the animal was apparently normal, but had lost a little weight.

#### EXPERIMENT No. 10.

An extract was made of the ash of 300 grams of the same plants as in experiment No. 9. The volume of the extract was 100 c. c.

December 4, 1909. Fifty cubic centimeters of this extract were fed at 11.30 a. m. to a rabbit weighing 2,170 grams; the remainder at 2.30 p. m., without serious ill effects. The next morning the animal was apparently well and weighed 2,050 grams. The calcium content of this extract was determined and proved to correspond to 8.5 grams calcium acetate.

#### EXPERIMENT No. 11.

December 11, 1909. An extract was made in the usual way of 500 grams *Astragalus mollissimus* Torr. (collection No. M. 9.45), from Hugo, Colo. The concentrated extract was made distinctly alkaline with sodium carbonate, pro-

ducing a heavy precipitate. The volume of the extract, precipitate and all, was 200 c. c. It was then centrifugated and the liquid poured off from the precipitate. The precipitate was suspended in water and about half of the suspension fed to a rabbit of 2,050 grams at 11.30 a. m. without much effect. At 4.30 p. m. the remainder was fed, but the rabbit was apparently in good condition the next day, weighing 2,000 grams.

December 12, 11.30 a. m. Fed 50 c. c. of the decanted liquid. In the early afternoon the animal was weak; at 4.30 p. m. very weak, not able to sit up or to hold head erect; lay flat on stomach; at 4.45 p. m. very weak; lay on its side, breathing rapidly; but heart action was good; nose and leg muscles twitched a good deal. At 5.10 p. m.: Convulsive movements of hind legs; an attack of opisthotonos. At 5.20: Opisthotonos for a few moments. At 6.20: Very feeble. At 8 p. m.: Dead in opisthotonos. Rapid onset of rigor mortis.

Autopsy: Stomach rather small; not very congested; contained gas; contents slight, containing a little mucus; mucous membranes only slightly congested; pylorus contracted. Right lung very congested, especially lower lobe. On cutting open the bronchi a clot was found in lower portion of right bronchus. As the animal had been lying on its right side in moribund condition from 6.20 p. m. to 8 p. m., the condition of the lung may have been hypostatic congestion, though there is the possibility of the beginning of a foreign-body pneumonia.

#### EXPERIMENT No. 12.

December 13, 1909. At 12 m. fed 50 c. c. of same liquid which had been decanted from the extract in experiment No. 11 to a rabbit weighing 1,225 grams. At 4.30 p. m. the remainder of the decanted liquid was fed. Not much effect. The animal died a week later of an acute febrile disease. At this time there was an epidemic among our rabbits. Hence the possibility that the rabbits of this experiment and of No. 11 died of disease must be considered.

#### EXPERIMENT No. 13.

December 22, 1909. A quantity of 250 grams *Aragallus lamberti* (Pursh) Greene was ashed and an acetic-acid extract made in the usual way. The *Aragallus lamberti* used contained barium. The extract had a volume of 55 cubic centimeters. It contained, as in the other cases, a certain amount of insoluble material in suspension and had an alkaline reaction. Fed 40 cubic centimeters to a rabbit weighing 1,512 grams without much effect. On December 23 the rabbit weighed 1,260 grams. The extract fed contained 8.5 grams calcium acetate.

#### EXPERIMENT No. 14.

December 29, 1909. An extract of 600 grams of *Aragallus lamberti* (Pursh) Greene (collection No. M 8.98) was made in the usual way. It was concentrated and made quite alkaline with sodium carbonate. The precipitate thus formed was removed by centrifugation, and half of it, suspended in water, fed to a rabbit of 1,260 grams. No visible effect.

December 31: Weight of rabbit 1,225 grams.

#### EXPERIMENT No. 15.

December 31, 1909. Fed 50 cubic centimeters of the liquid decanted from the precipitate fed in experiment No. 14 to a rabbit weighing 1,375 grams without producing any serious effect. The amount fed corresponded to about 250 cubic

centimeters of dry plant deprived of the precipitate formed by the sodium carbonate.

Experiments 14 and 15 were made because it was thought that there was some evidence that when the concentrated extracts were made alkaline with sodium carbonate most of the toxic material was precipitated. As this experiment shows, this idea was erroneous. By making alkaline, the calcium and probably any traces of barium that may have been present were precipitated as the carbonate. Calcium carbonate is but slightly if at all toxic. This treatment, viz, converting the calcium into an insoluble form, may have rendered the extract nontoxic. Experiments of a somewhat analogous nature are described in protocols Nos. 11 and 12.

#### EXPERIMENT No. 16.

November 8, 1909. At 12.15 p. m. fed 50 cubic centimeters of water containing in solution 2.5 grams anhydrous calcium chlorid to a white rabbit weighing 2,060 grams. No effects were noticed, and at 2 p. m. the dose was repeated. In all, 5 grams calcium chlorid were fed, or 2.94 grams per kilo of body weight. The rabbit lay on its side a while and urinated a good deal, but these were the only effects noted.

#### EXPERIMENT No. 17.

November 11, 1909. At 2.30 p. m. a brown rabbit weighing 1,915 grams was fed 48 cubic centimeters of a 9.5 per cent aqueous solution of anhydrous calcium chlorid, or 2.5 grams per kilo of body weight. The rabbit urinated as soon as it was put back into its cage; respiration was very rapid. At 3.50 p. m. the animal seemed normal. At 3.55 p. m. it urinated again. At 4.30 p. m. it still seemed normal. The dose was repeated, making the total amount of calcium chlorid fed 5 grams per kilo of body weight. At 5.45 p. m. the animal did not seem very sick; it did not eat, but was quiet.

The next morning the rabbit was found dead, lying flat on its belly, its hind legs drawn up under it as though it had collapsed without a struggle; body limp; no rigor; no bloating. On opening the abdomen some 25 to 50 cubic centimeters of serous fluid were found in the peritoneum. The intestines were not bloated. The stomach was large and prominent, the fundus being distended, congested, and very much reddened; the congestion of the fundus was greatest on the ventral side at the greater curvature, as though due to the accumulation in this place by gravity of the liquid fed. On opening the stomach it was found filled with partly digested food and considerable liquid; the mucous membrane of the ventral side in the region of the greater curvature of the fundus was filled with hemorrhages, eroded, and ulcerated; the pyloric end was nearly normal. The upper end of the small intestines was somewhat injected, but showed neither hemorrhages nor ulcers. The colon was also injected. The liver was congested. The heart was contracted. The lungs were normal.

#### EXPERIMENT No. 18.

November 24, 1909. At 11.30 a. m. a brown rabbit weighing 1,885 grams was fed 50 cubic centimeters of a 7.5 per cent aqueous solution of anhydrous calcium chlorid. There were no marked effects, so the dose was repeated at 5.15 p. m. Altogether, 3.98 grams calcium chlorid per kilo of body weight were fed. The second dose did not seem to affect the animal much. It seemed well the next morning and was eating. On November 26 it weighed 1,715 grams.

EXPERIMENT No. 19.

November 26, 1909. A brown rabbit weighing 1,715 grams was fed the extract prepared by digestion according to the method used by Crawford from 250 grams of *Astragalus nitidus* Dougl. collected at Woodland Park, Colo. The extract was administered in two doses on the same day, one in the forenoon, the other in the afternoon. Soon after the second dose the animal began to become weak and later had a few clonic spasms of the hind legs, which became partially paralyzed later, and the animal died about 6 p. m. without a struggle. On autopsy nothing was found except a highly inflamed and somewhat corroded condition of the fundus of the stomach.

EXPERIMENT No. 20.

October 20, 1909. A quantity of 225 grams of soy beans from Pittsville, Va., was ground and extracted in the usual way. The extract was concentrated on the water bath to a volume of 250 cubic centimeters.

Of this carefully neutralized extract, 75 cubic centimeters were fed at 4.30 p. m. to a rabbit of 2,010 grams. No effects whatever were noted. The next day at 9.30 a. m. 100 cubic centimeters were fed, with no effects whatever beyond some diuresis. The rabbit lost but very little weight. The total extract fed was equivalent to 157 grams of air-dried plant.

EXPERIMENT No. 21.

October 14, 1909. Fed 33 cubic centimeters of a colloidal solution of barium sulphate to a rabbit weighing 2,060 grams; no visible effect. October 15: Fed 50 cubic centimeters of the same solution; no effect. October 16: Fed 50 cubic centimeters of the same solution; no effect. In all, 6.5 grams barium sulphate were fed. The colloidal barium was prepared according to the method of Feilmann, casein freshly prepared from milk being used as the protective colloid.



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